UNIVERSITEIT GENT

Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen

Academiejaar 2000-2001

REACTOR START-UP AND PROCESS DESIGN FOR THE ANAEROBIC TREATMENT OF DOMESTIC WASTEWATER

REACTOR OPSTART EN PROCES ONTWERP VOOR DE ANAËROBE BEHANDELING VAN HUISHOUDELIJK AFVALWATER

door
ir. Youssouf KALOGO

Thesis submitted in fulfilment of the requirement for the degree of Doctor (PhD) in Applied Biological Sciences
Specialisation in Environmental Technology

Proefschrift voorgedragen tot het behalen van de graad van Doctor in de Toegepaste Biologische Wetenschappen
Specialisatie Milieutechnologie

op gezag van

Rector: **Prof. Dr. ir. J. WILLEMS**

Decaan:

Prof. Dr. ir. O. VAN CLEEMPUT

Promotor:

Prof. Dr. ir. W. VERSTRAETE

Ghent, 8 / 12 / 2000

The promotor:

Prof. Dr. ir. Willy Verstraete

The author:

ir. Youssouf Kalogo

In memory to my mother

Bamba Fatouman

1942 - 1990

There are no applied sciences there are only applications of sciences

Louis Pasteur

In the field of observation, the stroke of luck only favour the well prepared minds

Louis Pasteur

ACKNOWLEDGEMENTS

I express my gratitude to Prof. Willy Verstraete for giving me the opportunity to carry out my Ph.D. research in his laboratory.

I would like to thank the government of my country (Republic of Côte d'Ivoire) for providing me a grant for all these years of study in Belgium. This work would certainly not have reached its final stages without those financial supports.

I'm also grateful to Dr. Francis Rosillon and ir. Frederik Hammes for their spontaneous and free of charge co-operation in microbiological investigations.

I would like to thank Kris Graveel and Regine Haspeslagh, the two dynamic secretaries of the labMET, for their cordiality and assistance.

I'll always have a pleasant memory of the ten years sincerity relationship with my fellow countryman and colleague Arsène M. Séka.

I thank the members of the jury Prof. Agathos, Prof. Debevere, Prof. Lettinga, Prof. Van der Meeren, Prof. Vanrolleghem and Prof. Verloo for their critical comments and suggestions.

Last but not least, I thank my family for their continuing support. I specially thank my cousins Falikou Kallo and Amara Kalo, my younger brother ir. Daouda Kalogo and my engaged, Bara Djata Coulibaly for their encouragement in all these years.

Youssouf, 08 / 12 / 2000

NOTATIONS

AC-sludge	anaerobic control sludge
AH	anaerobic hybrid
ASB	anaerobic sludge bed
AW-sludge	anaerobic wemos sludge
BA	bicarbonate alkalinity (g $CaCO_3$ l^{-1})
BC	batch control reactor
BHI	brain heart infusion
BM	batch molasses addition reactor
B_v	volumetric loading rate (g COD l^{-1} d^{-1}or kg COD m^{-3} d^{-1})
BW	batch wemos reactor
C/N	carbon to nitrogen ratio
CEPS	chemically enhanced primary sedimentation
CEP-sludge	chemically enriched primary sludge
cfu	colony forming unit
CSTR	completely stirred tank reactor
CODt	total chemical oxygen demand (mg O_2 l^{-1})
CODs	soluble chemical oxygen demand (mg O_2 l^{-1})
CODss	suspended solids chemical oxygen demand (mg O_2 l^{-1})
CODs/VSS	soluble chemical oxygen demand to volatile suspended solids ratio
EGSB	expanded granular sludge bed
EPS	extracellular polysaccharides (µg uronic acid g^{-1}VSS)
FC	faecal coliforms (cfu 100 ml^{-1})
ΔG_0	Free energy (kJ $mole^{-1}$)
HRT	hydraulic retention time (h)
HUSB	hydrolysis upflow sludge bed
I.E.	inhabitant equivalent
Ks	half saturation constant (mg l^{-1})
MSW	municipal solids waste
NSFB	non-spore forming bacterium
TNK	total kjeldahl nitrogen
RACOD	rapidly acidifying COD

RC	control reactor
RE	relative expansion (%)
RFi	reactor with (100 - i)% of CEP-sludge from $FeCl_3$ pre-treatment
RM	molasses addition reactor
RNC	negative control reactor
RPC	positive control reactor
RW	wemos addition reactor
RWi	reactor with (100 - i)% of CEP-sludge from wemos pre-treatment
SAA	specific acidogenic activity (meq H^+ g^{-1}VSS h^{-1})
SEM	scanning electron microscopy
SMA	specific methanogenic activity (g COD-CH_4 g^{-1}VSS d^{-1})
SF	faecal streptococci (cfu 100 ml^{-1})
SFB	spore forming bacterium
SMSDW	synthetic mixture simulating domestic wastewater
SRB	sulfate reducing bacteria
SS	suspended solids (mg l^{-1})
TA	total alkalinity (g $CaCO_3$ l^{-1})
TC	total coliforms (cfu 100 ml^{-1})
TS	total solids (%)
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acid (mg l^{-1})
VFG-waste	vegetable fruit and garden waste
V_s	static settling velocity (m h^{-1})
VS	volatile solids (%)
VSS	volatile suspended solids (mg l^{-1})
VSS/SS	volatile suspended solids to suspended solids ratio
V_{up}	upflow velocity (m h^{-1})
WEMOS	water extract of *Moringa oleifera* seeds
Ybiomass	yield of biomass (g VSS formed g^{-1} CODt removed)
Y_{CH_4}	yield of methane (l CH_4 g^{-1} CODt removed)

CONTENTS

CHAPTER I

DEVELOPMENT OF ANAEROBIC SLUDGE BED (ASB) REACTOR TECHNOLOGIES FOR DOMESTIC WASTEWATER TREATMENT - MOTIVES AND PERSPECTIVES

Kalogo Y. & Verstraete W.

A modified version of this text was published as:

Kalogo Y. & Verstraete W. (1999) Development of anaerobic sludge bed (ASB) reactor technologies for domestic wastewater treatment - Motives and perspectives. *World Journal of Microbiology and Biotechnology* **15**, 523-534

CHAPTER I: DEVELOPMENT OF ANAEROBIC SLUDGE BED (ASB) REACTOR TECHNOLOGIES FOR DOMESTIC WASTEWATER TREATMENT- MOTIVES AND PERSPECTIVES

DEVELOPMENT OF ANAEROBIC SLUDGE BED (ASB) REACTOR TECHNOLOGIES FOR DOMESTIC WASTEWATER TREATMENT- MOTIVES AND PERSPECTIVES

1. INTRODUCTION

The upflow anaerobic sludge blanket (UASB) reactor developed by Lettinga *et al.* (1980) is often used currently for the anaerobic treatment of wastewaters (Hulshoff Pol *et al.*, 1997). This reactor was first introduced to optimise the anaerobic treatment of agro industrial wastewaters where it has proven its reliability (Pette & Versprille, 1981). The good results obtained for agro-industrial wastewaters coupled with the low investment and operation costs and the low sludge production and space required by the UASB, motivated sanitary engineers to apply it to the treatment of raw domestic wastewater with encouraging results. However, the reactor was confronted with some problems such as poor granular sludge formation, accumulation and slow hydrolysis of the suspended solids (SS), decrease in methanogenic activity and low biogas production. The problems of accumulation and slow hydrolysis of the SS and low biogas production motivated the upsurge of derived treatment technologies. These technologies are grouped under the name of anaerobic sludge bed (ASB) systems (Lettinga, 1996). In what follows, the ASB reactors developed to treat raw domestic wastewater are described and discussed. Then the purposes of the subsequent works, inspired by this literature review, are presented.

2. THE UASB REACTOR: OPERATION, PERFORMANCE, LIMITS AND POTENTIAL FOR DOMESTIC WASTEWATER

2.1 General operation and performance

The UASB is an anaerobic reactor fed from the bottom with an upflow velocity (V_{up}) of about 1 m h^{-1}. It can be divided into four compartments (from the bottom to the top): the sludge bed; the fluidised zone; the gas-liquid separator; and the settling compartment. In the sludge bed dissolved

substrate is readily digested, yet particulate matter firstly is intercepted then degraded through complex biochemical reactions (Figure 1).

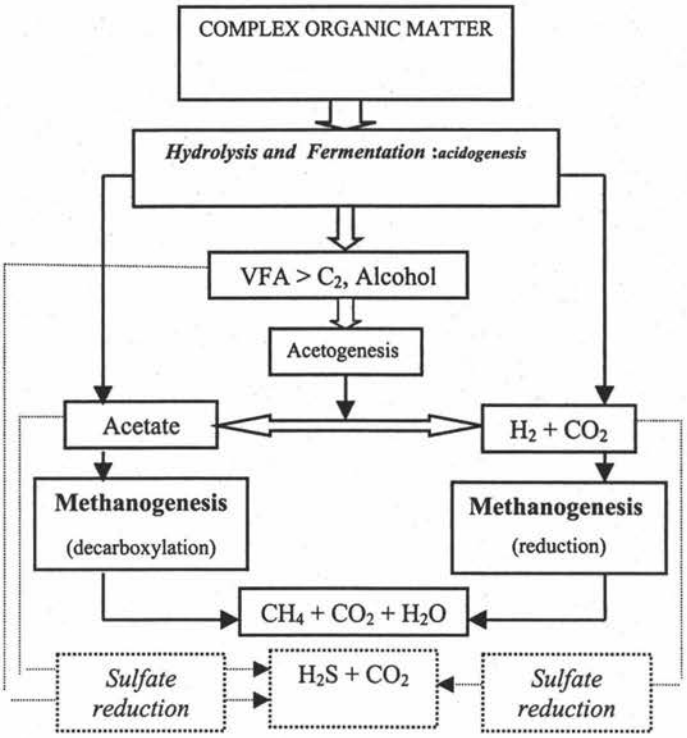


Figure 1. Anaerobic degradation pathway (modified and completed after Edeline, 1997a).

The particulate compounds present in the wastewater are hydrolysed and acidified to soluble compounds by extracellular enzymes secreted by the hydrolytic and acidogenic bacteria. These bacteria are extremely divers, strict or facultative anaerobes, e.g. *Clostridium*, *Klebsiella*, *Enterobacter*, *Erwinia*, *Streptococcus* etc and are generally also present in raw domestic wastewater (Crowther & Harkness, 1975; Britz *et al.*, 1994; Bitton, 1999). The products of their action on organic compounds are mainly volatile fatty acids (VFA),

hydrogen (H_2) and carbon dioxide (CO_2). The VFA are converted to acetate (CH_3COO^-) and H_2 by the acetogens, e.g. *Citrobacter*, provided the in-reactor H_2 partial pressure is lower than 10^{-4} atmosphere (Edeline, 1997a). In strict anaerobic conditions (E_0 < - 300 mV), CH_3COO^- and H_2, are converted to methane (CH_4) water vapour (H_2O) and CO_2. CH_3COO^- is transformed to CH_4 and CO_2 by the acetoclastic methanogens, mainly *Methanosarcina* and *Methanosaeta* (equation 1). Yet, *Methanosaeta* with a half saturation constant (K_s) of 42 mg l^{-1} out-competes *Methanosarcina* with K_s of 300 mg l^{-1} at low acetate concentrations (Brummeler *et al.*, 1985). This phenomenon seems also to occur at sludge retention time (SRT) above 30 days (Mah, 1983).

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \Delta G'_{01} = -35.9 \text{ kJ (mole)}^{-1} \qquad (1)$$

The H_2 and CO_2 formed during the hydrolytic and the acidogenic steps are transformed to CH_4 and H_2O by the hydrogenotrophic methanogens such as *Methanobrevibacter* and *Methanobacterium* (equation 2).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad \Delta G'_{02} = -135.9 \text{ kJ (mole)}^{-1} \quad (2)$$

The CH_4 and CO_2 are normally the most dominant gases of the biogas produced when sulfate (SO_4^{2-}) reduction is limited in the reactor. After passing the fluidised zone, the biogas and liquid are separated in the gas-liquid separator. The successful operation of the reactor requires a highly active biomass, e.g. 0.08 to 0.2 g COD-CH_4 g^{-1}VSS d^{-1} (Lettinga *et al.*, 1980), preferably aggregated in the form of granules with good settling properties while one may also have a working reactor with flocculent sludge bed. When the excess sludge is not discharged periodically, the sludge bed increases and the fluidised zone decreases. As a consequence, the excess sludge is washed out. The level at which the excess sludge washes out is called the maximum sludge hold-up.

Anaerobic digestion is considered to be possible under three ranges of temperature labelled psychrophilic, mesophilic and thermophilic. These temperature ranges, roughly represented in Figure 2, have no real boundaries. Many experiments have been done with the UASB technology, both at pilot and full scale, to treat raw domestic wastewater in a large range of

temperatures. The literature data summarised in Table 1 show that most of the pilot-scale reactors were operated at low temperature (7-19 °C). The total chemical oxygen demand (CODt) removal varied between 55 and 77% at the hydraulic retention time (HRT) of 4 to 72 h. These COD removal efficiencies were probably due to the SS removal, which was in the range of 55 to 94%.

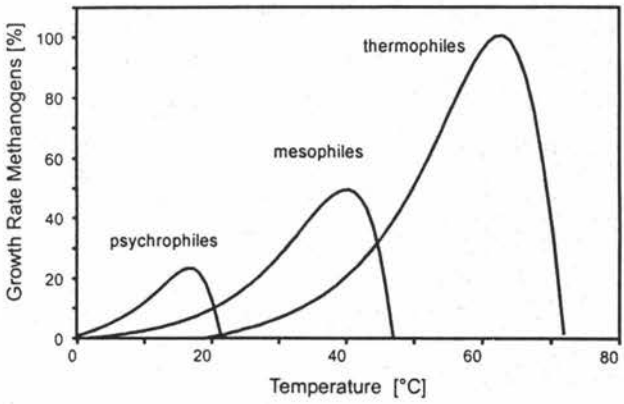


Figure 2. Relative growth rate of psychrophilic, mesophilic and thermophilic methanogens (after Wiegel, 1990).

Table 1. Anaerobic treatment of domestic wastewater with pilot-scale UASB reactors

V	T (°C)	V_{up}	Influent	Influent Concentration			B_v	Removal efficiency			Gas production		Inoculum	HRT	Startup	Authors
(l)		m h^{-1}	type	(mg l^{-1})			(kgCOD m^{-3} d^{-1})	(%)			l	l	type	(h)	-time	
				CODt	CODs	SS		CODt	CODs	SS	kg CODa	kg CODr			months	
120	7-12	+	RDW	200-1200	100-400	+	+	65	+	+	116-139	+	GS	8-12	+	Lettinga *et al.* (1981)
120	12-16	+	+	688	+	+	+	55-75	+	55-80	+	210	+	24	+	Lettinga *et al.* (1983)
118	19-23	+	RDW	460	+	+	+	60-75	+	+	115	+	none	24-40	+	Grin *et al.* (1983)
106	20-22	+	RDW	+	+	+	+	62	+	69	110	+	DS	4	+	Vieira & Souza (1986)
110	12-18	+	RDW	465	+	154	+	65	+	73	+	+	DS	12-18	+	Monroy *et al.* (1988)
106	35	0.7	PDW	100-500	+	50-250	+	65	+	61	118	+	DS	4	4	Vieira (1988)
120	19-28	+	RDW	627	151	376	+	74	52	72	80	+	none	4	4	Barbosa & Sant'anna (1989)
30	30	>.07	GEDW	600	+	+	0.4-6	90	+	+	+	150	various	24	2	Gnanadipathy & Polprasert (1993)
47.1	7-30	+	RDW	300	120	180	+	77	58	75	9.5-44.4*	+	DS	7-72	+	Agrawal *et al.* (1997)
400	+	+	RDW	684	225	251	2.7	88	81	92	+	+	+	6	**	Gonçalves *et al.* (1998)
416	+	+	RDW	640	239	202	+	77-87	69-77	75-94	+	+	+	4-6	+	Chernicharo & Machado (1998)
3.84	13	.075	PDW	344	124	82	+	59	45	79	+	+	GS	8	2	Elmitwalli *et al.* (1999)
3.84	13	.075	RDW	456	112	229	+	65	39	88	+	+	GS	8	1	Elmitwalli *et al.* (1999)

Note: RDW = Raw domestic wastewater; GS = Granular sludge; PDW = Pre-settled domestic wastewater; GEDW = Glucose enriched wastewater; DS = Digested sewage sludge; * =l m^{-3} wastewater treated; ** operated 18 months before; CODa = COD added; CODr = COD removed; + = Not indicated; V_{up} = Upflow velocity; B_v = Volumetric loading rate.

Table 2. Anaerobic treatment of domestic wastewater with full-scale UASB reactors

V (m^3)	T (°C)	V_{up} m h^{-1}	Influent type	Influent Concentration (mg l^{-1})			B_v (kgCOD m^{-3} d^{-1})	Removal efficiency (%)			Gas production l kgCODa	l kgCODr	Inoculum type	HRT (h)	Startup -time Months	Authors
				CODt	CODs (BOD_5)	SS		CODt	CODs (BOD_5)	SS						
64	25	+	RDW	+	+	+	+	78	(66)	75	+	+	+	6	+	Kooymans & Van Velsen (1986)
20	10-19	1	RDW	900	300	450	1.4-1.7	35-60	5-26	70-95	31-73	+	GS	13-14	+	De Man *et al.* (1988a)
35	23-24	+	RDW	430-520	(190-220)	200-250	1.95	66	(80)	69	+	+	+	5.2	+	Schellinkhout *et al.* (1988)
120	21-25	0.7	RDW	265-316	(128-151)	123-170	+	50-70	(61-71)	56-73	121-172	+	DS	4.7-9	+	Vieira (1988)
120	18-28	+	RDW	113-593	(50-253)	44-512	+	60	(70)	70	80-170	90-250	DS	5-15	+	Vieira & García (1992)
35	+	+	RDW	+	+	+	+	72-66	+	70-69	+	+	+	5-19	+	Schellinkhout & collazos (1992)
67.5	23	0.8	RDW	402	436	379	+	74	57	87	+	+	DS	7	5	Vieira *et al.* (1994)
2*3350	+	+	RDW	+	+	+	+	50-60	(68-73)	+	+	+	none	5.2	4-5	Schellinkhout & Osorio (1994)
477	+	+	RDW	600	+	303	1.25	68	+	76	+	+	DS	-	2	Chernicharo & Borges (1997)
1200	20-30	0.75	RDW	500	(200)	418	2.08	80	(77)	85	+	+	none	6	2-4	Tare *et al.* (1997)
12000	+	0.92	RDWT	+	+	+	5.625	24-50	(25-47)	29-73	+	+	none	8	2-4	Tare *et al.* (1997)
4800	+	0.61	RDW	+	+	+	1.10	49-65	(58-71)	50-76	+	+	none	8	2-4	Tare *et al.* (1997)

Note: RDW = Raw domestic wastewater; RDWT = Combined with tannery wastewater; GS = Granular sludge; DS = Digested sewage sludge; CODa = CODadded; CODr = COD removed; + = Not indicated; V_{up} = Upflow velocity B_v = Volumetric loading rate.

In contrast to the pilot-scale, most of the full-scale installations (Table 2) were operated at a temperature of 20-30 °C since these temperatures are more suitable for anaerobic digestion. Full-scale plants have been installed in tropical cities such as São Paulo and Saumare in Brazil (Vieira, 1988; Vieira *et al.*, 1994), Kampur and Mirzapur in India (Draaijer *et al.*, 1992; Tare *et al.*, 1997), Bucaramanga and Cali in Colombia (Schellinkhout & Collazos, 1992; Schellinkhout & Osorio, 1994) and Río Blanco in Mexico (Monroy *et al.*, 2000). The latter is currently the biggest UASB built in the world (volume = 83700 m^3 and capacity = 108000 m^3 d^{-1}). The plants were rarely operated at a HRT lower than 4 h but never exceeded a HRT of 20 h. The experience gained showed that under temperature conditions > 20° C, the COD removal efficiency of the reactors was directly related to the HRT (Figure 3).

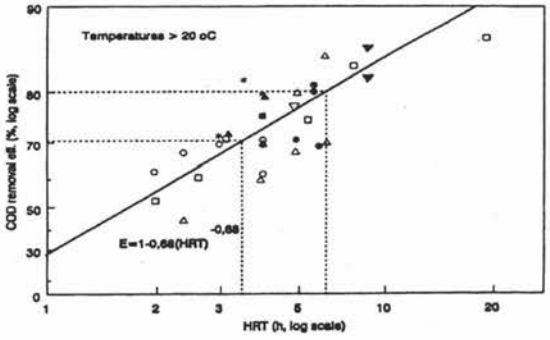


Figure 3. COD removal efficiency versus HRT at temperature > 20 °C (after Van Haandel & Catunda, 1997).

The higher the HRT, the better was the removal efficiency. However, a long HRT implies a large reactor volume. The problems related to the use of the UASB to treat domestic wastewater are the poor granular sludge formation, the accumulation and slow hydrolysis of the SS, the decrease in methanogenic activity and the low biogas production.

2.2. Reactor start-up and formation of granular sludge

During the installation of a UASB reactor, a proper inoculation can be a costly operation if good quality anaerobic granular sludge is not available on site. As raw domestic wastewater naturally contains some anaerobic and / or facultative anaerobic microorganisms, it may seem economical to start the reactor without inoculation. Different investigations on the self-inoculation of UASB reactors treating domestic wastewater have been reported (Tables 1 and 2).

Grin *et al.* (1983) were the first to study the self-inoculation of the UASB reactor during treatment of raw domestic wastewater. This study was done at a temperature of 19-23 °C and a HRT of 24-40 h. The COD removal efficiency was 60% after the first 4 months but gradually rose to 75% after 6 months. The results indicated that the start-up of a UASB treating raw domestic wastewater without inoculation was quite feasible. Later, Barbosa & Sant'Anna (1989) reported an excellent result of self-inoculation of a UASB reactor treating raw domestic wastewater. Operating at a HRT of 4 h and a temperature of 18 to 28 °C, they obtained granules 1mm in diameter one month after start-up. At the end of the 9-month operation, spherical granules up to 8 mm in diameter had developed and the COD removal efficiency was 74%. However, in none of the full-scale UASB reactors, built for the treatment of raw domestic wastewater and started without inoculation (Table 2), was granule formation observed. Despite this, the reactors were able to remove a CODt of up to 80%. The biomass grown in the reactor remained in the form of flocculent or fluffy sludge. Moreover, several researchers such as Gnanadipathy & Polprasert (1993), Wang (1994) and García *et al.* (1998) observed a poor inoculation. The latter observation demonstrates that the start-up and formation of granular sludge in a UASB reactor treating raw domestic wastewater without inoculation is a complex phenomenon. The excellent results reported by Barbosa & Sant'Anna (1989) were explained by the large amount of particulate COD (76%) present in the raw wastewater. It is however likely that the wastewater used by these researchers was very rich in both facultative anaerobic bacteria and substrate for the rapid growth of acidogenic bacteria. The importance of particulate COD coupled to the septic

character of the wastewater (concentration of microorganisms) was also supported by Lettinga *et al.* (1993). This implies therefore that self-inoculation of a UASB reactor will significantly improve if the retention of SS together with bacteria present in raw wastewater is enhanced through the reactor. This looks feasible when one refers to Grethlein (1978) who adopted a septic tank-membrane system to treat domestic wastewater. As a result of the increased concentration of microorganisms and substrate in the membrane reactor, the anaerobic digestion rate in the system was enhanced by a factor of 3-4.

Self-inoculation and, particularly, granular sludge bed formation still need further investigation since it is well known that the reliability of the UASB reactor is linked to the ability of bacterial cells to aggregate into granules (Lettinga *et al.*, 1983; Schmidt & Ahring, 1996). The main difficulty in granule formation during domestic wastewater treatment is currently attributed to the fact that prior to wastewater supply to the treatment plant, most of the rapidly acidifying COD (RACOD) is catabolised in the sewer (Verstraete & Vandevivere, 1999). In raw domestic wastewater, RACOD is in the range of 10-40 mg on a total of 210-740 mg COD l^{-1} (Henze *et al.*, 2000). Thus, the water entering the digester has insufficient substrate for acidogenic bacteria, which are essential for the growth of the granules (Thaveesri *et al.*, 1995a). The concept of granulation-enhancing additives or bio-supportive supplements was reported as a method to improve granule formation in UASB reactors. In this area, several additives (Table 3) were reported to be able to enhance granule formation.

Table 3. Supplements developed to improve granule formation in UASB reactors

Authors	Supplement (dose)	Substrate	Inoculum	Granule size (mm)
Kong *et al.* (1995)	RACOD (20% COD influent) + LABS (3-8 ppm)	VFA	30% Granular sludge and 70% Flocculent sludge	1-1.26
Wirtz & Dague (1996)	Acrylimide and Acryloxethyly - trimethylammonium chloride (1 mg l^{-1} reactor)	Sucrose	Anaerobically digested biosolids	≤ 2.3
Grootaerd *et al.* (1997)	Carrot pulp waste (20% total COD load)	VFA	Anaerobic sludge containing 30% granules	1-1.26
Imai *et al.* (1997)	Water adsorbing polymer (750 mg l^{-1} reactor)	Glucose and VFA	Anaerobic digested sludge	1.8-2.3
El-Mamouni *et al.* (1998)	Synthetic Percol 763 (2mg g^{-1}SS of reactor biomass) and natural Chitosan polymer (25mg g^{-1}SS of reactor biomass)	Sucrose	Syntrophic bacteria and disintegrated biomass	+

Note: LABS = Linear alkylbenzene sulfonate; VFA = Volatile fatty acids; RACOD = Rapidly acidifying COD; + = Not indicated.

Thaveesri (1995) demonstrated that a low liquid surface tension (<48 mN m^{-1}) can promote granules with a layered structure in which hydrophilic acidogens are predominant at the outer layer, provided that sufficient carbohydrate (e.g. sugar at a dose of 27% of COD of the influent) is present in the wastewater. The liquid surface tension can be lowered, by adding surfactants. Since these compounds are commonly present in domestic wastewater (Ahel *et al.*, 1994), the major limitation is the supply of energy rich substrate. Until now, no case of improvement, by supplement addition, of granulation in full-scale UASB reactors treating domestic wastewater is known and this challenge should be addressed. A totally different approach for the enhancement of granule formation in UASB reactors was reported by Wu *et al.* (1996). This approach is based on the presence of selective aggregate-forming bacteria such as *Methanosaeta*. There is indeed a general consensus, in the field, according to which these filamentous methanogens play an important role in granule formation (Wiegant & De Man, 1986; Dubourguier *et al.*, 1988; Hulshoff Pol *et al.*, 1988; Mac Leod *et al.*, 1990; El-Mamouni *et al.*, 1997).

Besides the biological aspects physicochemical factors such as calcium (Ca^{2+}) availability and engineering factors like hydrodynamics were also identified as influencing the process of granulation (Vanderhaegen *et al.*, 1992; Arcand *et al.*, 1994). Concentration of 50 mg Ca^{2+} l^{-1} is normally reached in domestic wastewater (Cisneros, 1995; Cisneros & Meíja, 1997). It is therefore unlikely that Ca^{2+} is the limiting factor of granulation on domestic wastewater. Most probably, attention should be paid to the hydrodynamic conditions and the supply of energy rich substrate as co-substrate.

It should be mentioned that one could import granular sludge from industrial reactors treating agro-industrial wastewater where granules are readily produced. Yet granular sludge has a market price of about 1.5 to 2.5 Euro per kg VSS. This cost can further increase when the transportation cost is taken into consideration. Such a granular biocatalyst will, when used to treat domestic wastewater, could fulfil it catalytic action but not tend to grow. Actually, one may therefore use a reactor with flocculent sludge since it can also provide better performances, as SS filtration might be better. Yet a flocculent sludge bed is easily washed out, when a high hydraulic load is applied, and this can cause system failure.

2.3. Effect of SS accumulation

The most important problem in the application of the UASB technology for domestic wastewater treatment is the presence of SS in the wastewater. Indeed, raw domestic wastewater contains about 45 to 55% of the COD in the form of SS (Tchobanoglous & Burton, 1991). According to Levine *et al.* (1985) particles can represent up to 85% of the total COD in domestic wastewater. The impact of the SS depends on the type of inoculum used (Sayed & Fergala, 1995). In a flocculent UASB reactor, the accumulation of SS present in the raw wastewater leads to an increase in the sludge bed height. When the accumulated sludge is not completely digested, it can lead to a dilution of the biomass. As a consequence, there is a gradual decrease in sludge activity. In a granular UASB reactor, the SS may affect the granules by forming a barrier around them. This barrier can lead to disintegration of the granules under long-term feeding conditions. The accumulation of the SS in

the reactor leads also to a decrease in COD conversion efficiency. These negative effects are particularly important when the reactor is operating at low temperature. However, even at temperature > 25 °C, the problem is not completely under control. For instance, in developing countries located in tropical regions one may be confronted with high concentration of poorly biodegradable SS in the wastewater especially during an intensive rain period. This is due to drainage of lands, waste and imperfection of sewerage systems.

Faced with this problem, it was established that the ratio of soluble COD to volatile suspended solids (CODs/VSS) of the wastewater should be > 10 to keep the anaerobic sludge sufficiently active for effective treatment (De Baere & Verstraete, 1982; De Baere *et al.,* 1984). Indeed, mathematical modelling has shown that otherwise the reactor volume tends to fill up with inactive suspended solids rather than with active microbial biocatalyst (Figure 4).

The control of SS has become important during the last decade. But efforts were mainly focused on Natural Primary settling (NPS) to remove SS before feeding some UASB reactors (Vieira, 1988; Elmitwalli *et al.*, 1998). This approach is simple and economically acceptable. However, it can take > 10 h to sediment the major part of the resistant fraction of the SS (Elmitwalli *et al.*, 1999). Currently, no investigation has been done in the line of increasing the CODs/VSS ratio. The design of a system dedicated to improve the CODs/VSS ratio of domestic wastewater before feeding it to the reactor must be the preferred option and may be a chemical pre-treatment system. Chemical pre-treatment can also remove efficiently colloidal particles at a very short HRT. The colloidal particles, although having a high biodegradability, are poorly removed in high-rate anaerobic reactors due to low physical removal probably because biomass and colloidal particles have both a negative charge (Elmitwalli, 2000).

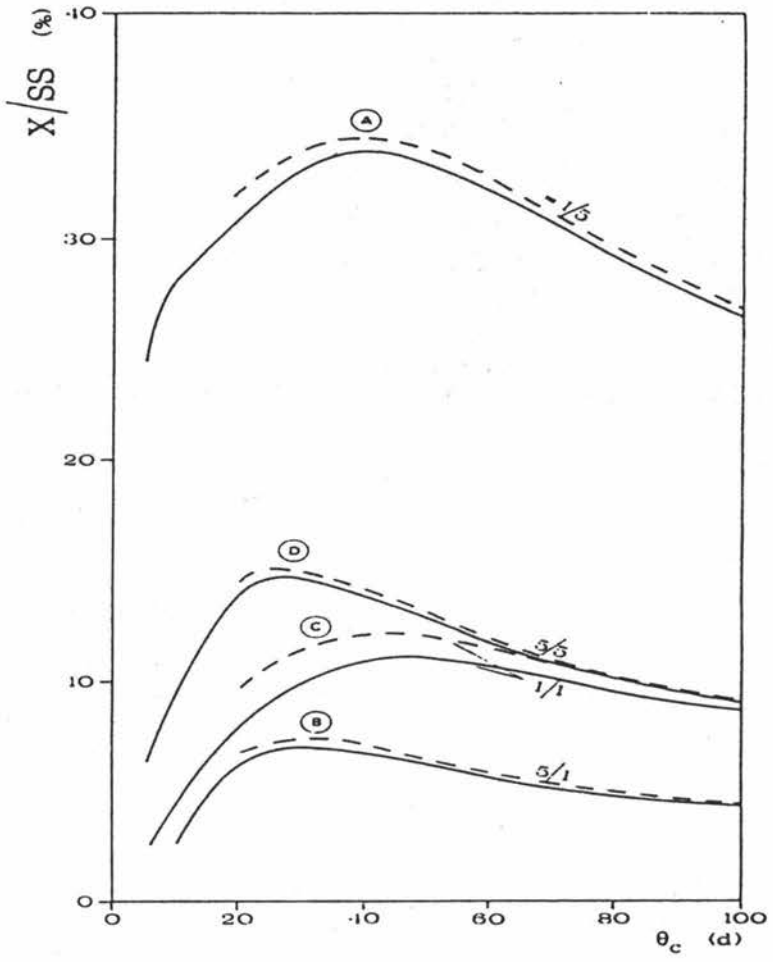


Figure 4. Ratio of biomass over sludge concentration (X / SS) versus SRT (θ_c) for different load conditions (after mathematical modelling by Rozzi & Verstraete, 1981): HRT = 0.5 day (continuous lines) and HRT = 20 days (dashed lines). The ratio's indicated on the curves A, B, C and D correspond to the VSS / CODs ratio. The figure indicates that at high VSS / CODs (low CODs / VSS) ratio the reactor tends to fill up with inactive SS rather than with active microbial biocatalyst (X).

2.4. Biogas production

Biogas and, particularly, CH_4 production are important aspects of the success of anaerobic digestion and cannot be disregarded during the anaerobic

treatment of domestic wastewater. Factors such as *low COD conversion efficiency*, presence of SO_4^{2-} and *wastewater strength* influence CH_4 production during anaerobic treatment of domestic wastewater. The COD removal efficiency is calculated on the basis of influent COD minus effluent COD divided by influent COD. The operation of the reactor is very close to that of a plug flow reactor (Brito & Melo, 1997). In some instances a good COD removal efficiency can be observed, while the conversion efficiency to CH_4 is low. Such a removal may be due to interception of the SS in the sludge bed (Van Haandel & Lettinga, 1994). The low COD to CH_4 conversion efficiency results from the slow hydrolysis and acidification of the SS accumulating in the reactor.

The concentration of SO_4^{2-} present in domestic wastewater can vary from 50 to 200 mg SO_4^{2-} l^{-1} (Yoda *et al.*, 1987). In a temperature range of 30 to 35 °C, sulfate-reducing bacteria (SRB) are able to oxidise a part of the COD (mostly via the H_2-intermediary state) present in the wastewater by utilising SO_4^{2-} as an electron acceptor as shown by equation 3 (Widdel, 1988).

$$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + H_2O \quad \Delta G'_{03} = -150 \text{ kJ (mole)}^{-1} \quad (3)$$

At higher temperatures (e.g. 55 °C), SRB can compete with acetoclastic methanogens for acetate as indicated by equation 4 (Visser *et al.*, 1992).

$$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^- \quad \Delta G'_{04} = -71 \text{ kJ (mole)}^{-1} \quad (4)$$

In both cases, the product formed is H_2S when HS^- reacts with H^+ available in the liquid phase. With high concentration of SO_4^{2-} little CH_4 results since a greater electron flow goes towards SO_4^{2-} reduction (Harada *et al.*, 1994). SRB grow over a wide pH range (5 – 9) which includes the optimum range (7.0 – 7.5) for the methanogens and SO_4^{2-} reduction to proceed requires low energy than methanogenesis ($\Delta G'_{03}$ and $\Delta G'_{04}$ compared to $\Delta G'_{02}$ and $\Delta G'_{01}$ respectively). This means that SO_4^{2-} reduction cannot be fully kept under control in methanogenic reactors unless SO_4^{2-} is not available.

The wastewater strength (biodegradable COD concentration) has a direct effect on the production of CH_4 during anaerobic digestion. Figure 5 shows the influence of the biodegradable COD concentration on the production of

CH_4 at ambient temperature. A part of the CH_4 produced remains constantly dissolved in the effluent. Also, there are some losses related to imperfections in the collector systems. Thus, for COD concentrations < 300 mg l^{-1} the volume of CH_4 collected per cubic metre of influent treated is quite low (< 50 ml CH_4 l^{-1} influent).

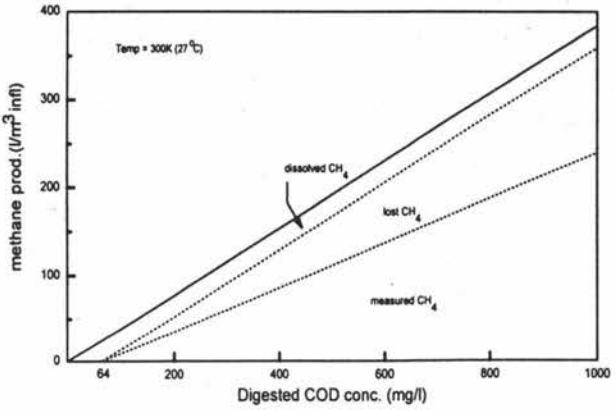


Figure 5. CH_4 production, release to the gas phase and CH_4 collected as a function of the digested COD concentration (after Van Haandel & Lettinga, 1994).

Because of the above, it has been observed frequently that treatment of domestic wastewater in a UASB reactor produces, per kilogram of COD removed in the reactor, a lower volume of CH_4 than the expected value of 350 l CH_4 kg^{-1}COD removed. Some experimental values observed are 210 (Lettinga *et al.*, 1983), 37.5 – 80 (Draaijer *et al.*, 1992), 190 (Lettinga *et al.*, 1993) and 150 l CH_4 kg^{-1}COD removed (Gnanadipathy & Polprasert, 1993). In many cases, it seems uneconomical to use the CH_4 produced as a fuel and, therefore, it is generally vented via a chimney or flared off (Van Haandel & Lettinga, 1994). This practice can create a negative image of anaerobic processes since CH_4 and CO_2 are the most important greenhouse effect gases. Low biogas production can also affect the reactor performance since it cannot

provide the high hydraulic mixing intensity, which is necessary for good treatment of diluted wastewaters such as the domestic wastewater. This presumes that more energy is required to maintain a better hydrodynamic condition in the reactor.

Despite the low gas production, UASB technology is more and more preferred for the treatment of domestic wastewater rather than the conventional aerobic systems, which were widely used in the past. The main reasons for this are the low investment, operation and sludge disposal costs and low space requirement for the UASB as shown in Table 4.

The limits related to the UASB reactor, when used to treat domestic wastewater, have led to the emergence of different anaerobic sludge bed systems.

Table 4. Land surface required and costs estimated for different domestic wastewater treatment systems (after Vieira & Garcia, 1992; Mergaert *et al.*, 1992; Schellinkhout, 1993; Van Haandel & Catunda, 1997)

Type of process	Type of treatment	Land surface required (m^2IE^{-1})	Costs for 100,000 IE (Euro IE^{-1} $year^{-1}$)		
			C	O	SD
Aerobic	Oxidation ditch	0.2	15	15-20	2-4
	Trickling filter	0.54	15	15-20	2-4
	Activated sludge	0.2-0.4	15	15-20	2-4
	Waste stabilization pond	2-4	+	+	+
Anaerobic	UASB	0.015-0.05	7.5	7.5-10	1-2

Note: C = Capital; O = Operation; SD = Sludge Disposal; + = Not indicated; IE = Inhabitant Equivalent.

3. ALTERNATIVE ASB REACTOR SYSTEMS

3.1. The expanded granular sludge bed (EGSB) reactor

The EGSB reactor was the first modified form of the UASB reactor. This reactor concept was introduced by De Man *et al.* (1988b) to increase the

hydraulic mixing intensity, which is low in the UASB reactor. The EGSB reactor is characterised by an expansion of the granular sludge bed. This expansion is due to the application of a high V_{up} (5-10 m h^{-1}) which is 5 to 10 times higher than that of a UASB reactor. The EGSB reactor behaves as a completely stirred tank reactor (CSTR) due to the effect of the high V_{up} (Brito & Melo, 1997). The high velocity was found to improve the contact between the wastewater and the biomass (De Man *et al.*, 1988b). Indeed it decreases the apparent K_s of the granular biomass (Kato *et al.*, 1997). This makes the EGSB reactor a system particularly suitable for loading conditions < 1 kg COD m^{-3} d^{-1} (Lettinga *et al.*, 1993). In the EGSB reactor the interception of the SS in the granular sludge bed is decreased because they are washed out (Vander Last & Lettinga, 1992). As a consequence, the quality of the effluent from a EGSB reactor is lower than that of a UASB reactor (De Man *et al.*, 1988b). To date, the use of the EGSB for the *direct* treatment of raw domestic wastewater has been limited to the reports of De Man *et al.* (1988b) and Vander Last & Lettinga (1992). The results reported did not demonstrate the true potential of this reactor. The COD removal efficiency of the reactors was low (30 to 40%), although they were inoculated with granular anaerobic sludge. However, the EGSB reactor has been shown to be reliable for other types of wastewaters. As demonstrated by Kato *et al.* (1994), the EGSB concept is very attractive for low-strength wastewater (biodegradability > 70 %) even under psychrophilic conditions (Rebac, 1998). It also seems to be promising for wastewater containing oleic acid provided the HRT is prolonged to 24 h and glucose or butyrate is supplied as a co-substrate (Hwu, 1997). It has also been reported to be feasible for the treatment of wastewater containing ethanol with a COD concentration as low as 13 mg l^{-1}, facilitating a removal efficiency of 85% (Kato *et al.*, 1997). In this respect, the EGSB reactor should serve as a robust polishing reactor that is comparable to aerobic systems. Such a capability of the EGSB reactor has not yet been demonstrated for domestic wastewater treatment. Most probably, the ever-increasing demands for combined removal of COD and SS curtail interest in the EGSB for the direct treatment of domestic wastewater. It is however a pity that no attempts have been made in the line of direct development of granules, on

domestic wastewater, with EGSB reactor although it may offer favourable hydraulic stress conditions.

3.2. The UASB-septic tank

The originality of the UASB-septic tank compared to the conventional UASB reactor is that the former includes accumulation and stabilisation of the sludge. It also differs from the conventional septic tank in the fact that it operates in upflow mode. In practice, the UASB-septic tank operates as a continuous system with respect to the liquid as well as a batch system with respect to the solids. This reactor concept for the treatment of domestic wastewater was first investigated by Bogte *et al.* (1993) in the Netherlands. At low temperature conditions (12-14 °C), the COD removal efficiency was comprised between 33 and 60% at a HRT of 44 to 102 h. Lettinga *et al.* (1993) investigated the feasibility of this system in Indonesia in a 0.86 m^3 reactor. Under tropical conditions, the results were more interesting. The COD removal efficiency was 67 and 93% at a HRT of 34 hours (black + grey water) and 360 hours (black water) respectively. The influent COD was, respectively, 1.35 g l^{-1} and 5.5 g l^{-1} for each type of water. The long HRT generally applied for the UASB-septic tank implies a low hydraulic load. But the sludge hold-up time of the system is so long that sludge discharge is only required once every three to four years. In a classical UASB, due to the short HRT, the hydraulic loading rate is higher. Thus, the high hydraulic load considerably shortens the sludge hold-up period of the reactor. This requires the classical UASB to discharge frequently (once or twice a week) the excess sludge produced. The discharged sludge needs to be further stabilized in a separate reactor. Chen (1996) demonstrated that the addition of 10 mg polyelectrolyte (PE) per litre of raw wastewater treated in a UASB-septic tank can facilitate a COD removal efficiency of 80% at a HRT of 17 h and a temperature of 22 °C. The probable impacts of the PE dosage on the excess sludge hold-up and its stabilisation are not yet clear.

The long HRT necessary for the operation of the UASB-septic tank makes it unsuitable as a common treatment system in densely populated areas. Indeed,

in such a situation the system will need a large volume of ca 0.3 - 3 m^3 reactor per I.E treated. This system appears to be potentially useful in rural areas where it is uneconomical to build sewers and conventional treatment plants. The fact that the sludge discharge is required only once every three to four years means that biomass with a high hydrolytic activity to hydrolyse the SS is maintained in the bioreactor. However, the system still needs further investigation since very little experimental data are available to confirm its reliability.

3.3. The hydrolysis upflow sludge bed (HUSB) reactor

The HUSB reactor differs from the UASB reactor by the absence of a three-phase separator, which is an important aspect of the design of the latter. Moreover, the content of the reactor is slightly stirred so that the sludge is kept in suspension. The HUSB reactor concept for raw domestic wastewater treatment was proposed in 1985. It was first tested at pilot-scale (170 m^3) at the Gao Beidian (China) wastewater treatment plant as reported by Wang (1994). The system achieved a very satisfactory SS removal (75 – 84%) at a short HRT (2.5 – 5 h) and ambient temperature (18 – 31°C). However, the total COD removal of the system was only 40 – 48% and the soluble COD removal was very low. The volumetric loading rate was ≤ 5 kg COD m^{-3} d^{-1}. The results showed that the system only effected a pre-acidification. This is an important feature of the reactor since acidification is sometimes the limiting step of the anaerobic process.

The mechanisms for pollutant removal by the HUSB reactor seem to be similar to those operating in a primary sedimentation tank. However, sometimes, refractory molecules can be converted into readily degradable compounds in the HUSB reactor (Wang, 1994). Several full-scale installations have been built in China since 1992. The first installation was built in Changji. It had a volume of 630 m^3 and a capacity of 12,000 – 15,000 m^3 d^{-1}. The COD removal efficiency was 18% at an HRT of 2.5 h when the reactor was started without seeding but reached 52% removal after addition of a concentrated activated sludge as seed and an HRT increase from 2.5 to 4 h.

Five other installations were built in different cities (Miyun, Anyang, Shenzheng, Wuifang and Kunming) with capacities which varied from 3,000 to 20,000 $m^3 d^{-1}$. The HUSB reactor can be considered as a system that can be coupled to the UASB reactor for the treatment of raw domestic wastewater (Wang, 1994).

3.4. The two-stage reactor concept

Very often, there is confusion between the expressions: two-phase and *two-stage or two-step* reactor. The former implies a process configuration employing separate reactors, for acidification and methanogenesis, connected in series. The latter is designed so that acidification in the first reactor is incomplete (Wang, 1994). The main advantages of the two-phase system appear to be improvements in process control and low accumulation of biomass in the methanogenic phase (Verstraete *et al.*, 1981). However, the system has some disadvantages. In particular, phase separation affects interspecies H_2 transfer from acidogens to methanogens in a digesting system (Burel & Trancart, 1985). The two-phase concept has become obsolete. The preference is for the *two-stage* concept because complete acidification, as it occurs in the two-phase reactor, can slow down the granulation process and influence negatively the performance of the second reactor (Lettinga & Hulshoff Pol, 1991). Based on the UASB technology, at least two types of *two-stage* reactors were proposed for raw domestic wastewater treatment. The results from different studies are summarised in Table 5. The total COD removal efficiencies of these systems varied in the range of 70 to 80%. Based on the data currently available the *two-stage* reactors have a better performance, than the *one-stage*, although working at much shorter HRT (Table 5).

Table 5. Results of anaerobic treatment of domestic wastewater in *two-stage* reactors compared to *one-stage* reactor

Parameter	Two-stage Sayed & Fergala (1995)	Two-stage Wang *et al.* (1997)	One-stage Pilot-scale Average Table 1	One-stage Full-scale Average Table 2
Process type	UASB-UASB	HUSB-UASB	UASB	UASB
Volume (m^3)	0.042 (0.0046)	200 (120)		
Temperature (°C)	18-20	17	19 ± 8	22 ± 5
HRT (h)	8-4 (2)	3 (2)	16 ± 12	9 ± 4
Influent				
CODt (mg l^{-1})	200-700	650	519 ± 266	464 ± 216
CODs (mg l^{-1})	+	+	184 ± 102	368 ± 96
SS (mg l^{-1})	90-385	217	197 ± 97	285 ± 153
B_v (kgCOD m^{-3} d^{-1})	1.22-2.75 (1.70-6.20)	5.3 (4.0)	3.03 ± 2.81	2.16 ± 1.6
efficiency (%)				
CODt	74-82	69	71 ± 11	60 ± 15
CODs	73-100	79	60 ± 16	29 ± 26
SS	86-93	83	76 ± 12	70 ± 15

Note: ( ) = Data in brackets relate to the second reactor; B_v = Volumetric loading rate; + = Not indicated.

Wang (1994) and Wang *et al.* (1997) suggested the combination of the HUSB reactor and the EGSB reactor. The latter study was done at pilot-scale in a 200 l HUSB and a 120 l EGSB. During the study, the sludge was discharged daily from the HUSB reactor and fed once a day to a separate sludge recuperation tank, with a 2-day retention time, where it was digested anaerobically for stabilisation (Figure 6).

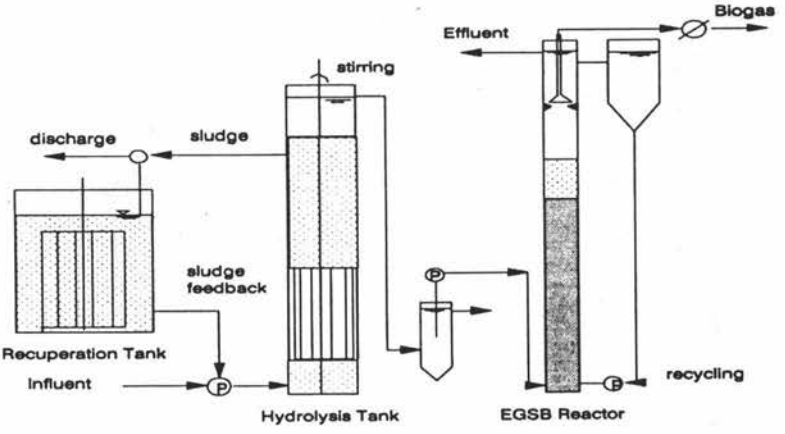


Figure 6. Schematic representation of a two-stage reactor (HUSB-EGSB) combined with a sludge stabilization reactor (after Wang, 1994).

Sayed & Fergala (1995) studied a pilot-scale *two-stage* system, which consisted of three UASB reactors. The first stage had two identical flocculent sludge UASB reactors. These reactors were used to remove and digest the SS through an intermittent mode of operation. During the feeding of one reactor, the other remained unfed for the stabilisation of the organic solids accumulated during its feeding period. The second stage reactor was a granular sludge UASB reactor which received the effluent free of particles from the first-stage working reactor.

The *two-stage* reactor concept seems particularly attractive. However, there is a real need for regular discharge of the excess sludge from the first reactor. The necessity of introducing a third reactor can increase the investment and operation costs of the treatment plant. It can also make it more complicated technically. This can probably explain why such full-scale installations are not yet operating for raw domestic wastewater treatment.

3.5. The anaerobic hybrid (AH) reactor

The AH reactor is a combination of a UASB reactor or a EGSB reactor and an anaerobic filter (AF) in one reactor. The reactor bottom is a sludge bed and the top is a filter on which biomass can be attached. Although eight hybrid reactor plants have been built in Mexico since 1997 (Monroy *et al.*, 1997), literature data about this system are still scarce. Recently, Elmitwalli *et al.* (1999) studied an AH reactor for the treatment of raw and pre-settled domestic wastewater under low temperature conditions (13° C) using small sludge granules instead of large sludge granules. Operating at a HRT of 8 h, the AH reactor removed 66% of the CODt and 92% of the suspended solids COD (CODss) from the raw wastewater and 61% of the CODt and 87% of the CODss from the pre-settled wastewater. The main advantages of the AH system seem to be related to the prevention of sludge flotation and the good removal of SS. However, some improvements appear to be necessary to avoid the formation of channels and gas pockets in the sludge bed.

4. BOTTLENECKS

To date, the experiences gained with ASB reactors for the treatment of raw domestic wastewater may be summarised in relation to their specific process parameters and their main advantages and disadvantages (Table 6).

Table 6. Specific process parameters, main advantages and drawbacks of ASB reactors treating raw domestic wastewater

Reactor configuration	B_v (kg COD m^{-3} d^{-1})	HRT (h)	Sludge discharge regime	Main advantage	Main drawback
UASB	1–3	4–20	1 to 2 times per week	Good interception of SS	Slow degradation of SS
EGSB	≤ 1	2–3	(*)	Low apparent K_s value	Wash out of SS
UASB-septic tank	0.4-1	30–350	Once every 3 or 4 years	Self stabilization of the excess sludge	Less efficient for total wastewater treatment (black + grey)
Two-stage and single HUSB	1-5	5–10	Daily	Good acidification of SS	External reactor for excess sludge stabilization
AH reactor	> 1	4-8	(+)	Prevention of sludge flotation and wash out	Channels and gas pockets in the sludge bed

Note: (*) = Not indicated in the literature, due to the higer V_{up} it should be more frequent compared to the UASB; (+) = Not indicated in the literature.

Together with the advantages and disadvantages specific to each system presented, the reactors have common disadvantages with regard to their potential for complete treatment. For example, these systems do not effect nutrient and pathogen removal. Hence, they are generally combined with a subsequent aerobic system for post-treatment. Such a combination is presumed to be more economical than full aerobic treatment (Pipyn *et al.*, 1994). In full-scale applications, facultative lagoons and oxidation ponds were used respectively in Kampur (India), Itabira (Brazil) and Bucaramanga (Colombia) for polishing UASB reactor effluent. Several newer applications

were also tested at pilot-scale or laboratory-scale for nitrogen removal. A combined UASB and sequencing batch reactor system, for the removal of nitrogen, was proposed by De Sousa & Foresti (1996). This combination resulted in very low excess sludge production (4% of the influent COD). A combination of a UASB and an aerated biofilter was suggested by Gonçalves *et al.* (1998). Another combination of the UASB reactor and the hanging sponge cubes process for nitrogen removal was investigated by Agrawal *et al.* (1997). A rotating biological contactor combined with a UASB reactor was introduced to treat domestic sewage in coastal areas (Castillo *et al.*, 1997). In some situations, nutrient removal is not of concern since the effluent from the anaerobic digester can be a valuable product for irrigation and fertilisation (nitrification is carried out by nitrifying bacteria present in the soil) provided that the pathogens are removed (Zeeman & Lettinga, 1999). This is, typically, the case in tropical areas where the availability of nutrients and sufficient water for agriculture is of prime importance (Dixo *et al.*, 1995; Shereif *et al.*, 1995; Cisneros, 1995; Cisneros & Mejia, 1997). Fortunately, waste stabilisation ponds effectively remove pathogens from UASB reactor effluent (Dixo *et al.*, 1995; Catunda & Van Haandel, 1996). Recently, Van Der Steen *et al.* (1999) proposed a system which combined duckweed ponds and waste stabilisation ponds to achieve a better removal efficiency. In the case of land limited conditions, a very compact system would be preferred. Some chemical methods such as chlorination have been examined as an alternative for the disinfection of UASB effluent. Unfortunately, chlorinated effluent very often has a negative impact on irrigated crops (Takaski, 1998).

From this review, it appears that so far the UASB reactor has been widely applied to treat domestic wastewater. Although encouraging results were obtained various problems were also observed. Overall, investigations on self-inoculation suggest that the process may need to be optimised in view of the slow start-up. In order to guarantee a stable system, effort on the line of promoting granules should be increased. Accumulations of SS lower biomass activity and affect negatively the COD to CH_4 conversion efficiency. The low biogas production can therefore not provide good mixing in the reactor and the small volumes of biogas can also not be used efficiently. Facing the

problems caused by the accumulation of SS various engineering efforts have been done for some years to develop new reactor concepts.

In an EGSB reactor, the application of an upflow velocity of 5-10 m h^{-1} decreases the interception of the SS in the sludge bed, therefore the effluent quality tends to decrease. The previous experiments did not demonstrate the true potential of this reactor.

The UASB septic tank needs very long HRT's for proper operation. It is therefore unsuitable as common treatment system in densely populated areas.

The two-stage reactor concept is attractive. But the necessity of introducing a third reactor for the stabilisation of sludge discharged from the first reactor can increase the investment and operation cost and also the technical problems.

Finally, the proposal of Monroy *et al.* (2000) that a proper integration of anaerobic digestion systems for instance for water reuse and energy recovery is needed in many countries deserves further support. The economic niche for anaerobic digestion is so large and challenging that sanitary engineers have to increasingly step up their efforts to provide an appropriate technology for the treatment of domestic wastewater.

5. HYPOTHESES

H_1. Self-inoculation of a UASB reactor will significantly improve if the retention of suspended solids together with bacteria present in raw domestic wastewater is enhanced inside the reactor.

H_2. Development of granular sludge on domestic wastewater will be possible if favorable hydrodynamic conditions are applied or an energy rich substrate is co-supplied to the reactor.

H_3. High-rate anaerobic treatment of domestic wastewater will be feasible if the reactor is retrofitted by a chemically enhanced primary sedimentation (CEPS) treatment.

6. OBJECTIVES AND SCOPE OF THE THESIS

This work presents the results of research activities conducted at laboratory scale on domestic wastewater. The purpose of the research was to solve the above mentioned problems related to the anaerobic treatment of domestic wastewater. Subsequently, the basis of the feasibility of an integrated concept for the treatment of domestic wastewater was established. The research was therefore focused both on scientific and engineering processes as described by the three hypotheses previously formulated. The investigation was initially focused on reactor start-up (Chapter II, III, IV and V), then on process design (chapter VI and VII).

Chapter II, III and IV deal with the understanding and the enhancement of the self-inoculation of UASB reactors. The effects of a natural polymer, the water extract of *Moringa oleifera* seeds (WEMOS), on shortening the reactor start-up are presented.

Chapter V compares the effects of reactor hydrodynamics and addition of co-substrate, e.g. molasses, on promoting granular sludge on domestic wastewater.

Chapter VI describes the feasibility of a tandem CEPS-UASB reactor for the treatment of raw domestic wastewater.

Chapter VII explores the co-digestion of primary sludge, from the CEPS-UASB treatment, with vegetable fruit and garden (VFG)-waste. This investigation focused on the buffer capacity of the waste-sludge to stabilize the anaerobic digestion process of VFG-waste.

Finally, based on the results obtained, an integrated concept for the anaerobic treatment of domestic wastewater is proposed in Chapter VIII. This final Chapter concludes the work done and comments on further application of the suggested concept.

CHAPTER II

START-UP OF A SELF-INOCULATED UASB REACTOR TREATING DOMESTIC WASTEWATER - PART I. PROCESS DYNAMICS

Kalogo Y. & Verstraete W.

A modified version of this text was accepted for publication as:

Kalogo Y., Mbouche J.M. & Verstraete W. (2000) Physical and biological performance of a self-inoculated UASB reactor treating raw domestic sewage. *Journal of Environmental Engineering* (in press)

START-UP OF A SELF-INOCULATED UASB REACTOR TREATING RAW DOMESTIC WASTWATER - PART I. PROCESS DYNAMICS

Abstract - The dynamics of a self-inoculated upflow anaerobic sludge blanket (UASB) reactor treating raw domestic wastewater has been studied. The results suggested two types of start-up periods. The first 6 weeks of operation, the reactor achieved a removal efficiency up to 65% of total chemical oxygen demand (CODt) and 73% of suspended solids (SS). During this primary start-up period, the removal of organic matter was mainly due to interception in the reactor. The second start-up period expressed by biogas release was reached after 14 weeks. An intermediary period between these two start-up periods was characterised by a slight drop of pH and increase of volatile fatty acid (VFA) in the effluent. The long time to reach the second start-up period seemed to be responsible for the large discrepancy between CODt removed and biogas recovered (107 ml g^{-1} CODt removed). It is thus suggested that process optimisation should focus on shortening the time needed to reach the second start-up period. A model that represents a possible description of the process dynamics of self-inoculated UASB reactors is proposed.

Key words: Anaerobic digestion, domestic wastewater, UASB reactor, self-inoculation.

INTRODUCTION

Anaerobic treatment in the upflow anaerobic sludge blanket (UASB) reactor, developed by Lettinga *et al.* (1980), is a process of increasing importance for purification of domestic wastewater. As all biological treatments, this process is based on the natural self-purification capacity of rivers, which itself is linked to the actions of the autochthonous microbial communities. Yet, in order to shorten the start-up period and guarantee high purification efficiency, reactors are often inoculated "manually" with high amounts of active microorganisms in the form of

granules.

Since the last decade, the ever-growing demands to treat domestic wastewater in UASB reactors and the difficulties to obtain good and cheap granular sludge have prompted researchers to investigate self-inoculation. This method appeared attractive in view of the presence of both anaerobic and facultative anaerobic microorganisms in raw domestic wastewater (Crowther & Harkness, 1975; Bitton, 1999).

Earlier pilot-scale experiments conducted by Grin *et al.* (1983) and Barbosa & Sant'Anna (1989) were rapidly followed by several full-scale installations (Draaijer *et al.*, 1992; Lettinga *et al.*, 1993; Schellinkhout & Osorio, 1994; Tare *et al.*, 1997) in different regions. The reports from the pilot-scale and full-scale installations were mainly focused on the process performance. The performance of these studies showed a large discrepancy between the total COD (CODt) removal and the biogas recovery rate, which was generally explained by interception of organic matter in the reactor. But the process dynamics in relation to physical and biological performance of self-inoculated reactors was not mentioned in detail and there is still a lack of knowledge in this area. These aspects are, however, essential to understand how the discrepancy between CODt removal and biogas recovery can be explained. Moreover they can provide useful insights for better monitoring, control and eventually for optimisation of the process performance. Indeed, the most recent reports have shown that self-inoculation may need to be optimised in view of the slow start-up (Gnanadipathy & Polprasert, 1993; Wang, 1994; Garcia *et al.*, 1998).

The present study was therefore carried out at laboratory scale to explore further how the process occurs during the operation of a self-inoculated UASB reactor treating raw domestic wastewater. The main objective of this investigation was to understand the dynamics of the reactor in terms of physical and biological performance during its start-up period.

MATERIALS AND METHODS

Operation of the UASB reactor

A laboratory UASB reactor operating using the parameters summarised in Table 1 was set up without any special inoculum and fed continuously for 22 weeks with raw domestic wastewater (Table 2) obtained from the Ossemeersen domestic wastewater treatment plant, city of Ghent (Belgium). The volatile fatty acid (VFA) content of the feed was low c.a. 4% of the CODt. The COD in the form of SS was 56% of the CODt.

The reactor was made of glass (Schott-Duran, Germany) with a height of 90 cm and an internal diameter of 5 cm. On top, a decantation sphere of a 0.5 l volume was fitted. The reactor was fed by using a peristaltic pump (Watson Marlow, 313S, Germany). The top of the reactor was connected to a gas column and the pH of the liquid in the gas column was lowered to 4, by adding HCl (Vel, Leuven, Belgium) in order to avoid the dissolution of CO_2 of the biogas. Methyl orange (Vel, Leuven, Belgium) was added to the liquid as indicator of the biogas level in the column.

Table 1. Operating parameters of the UASB reactor

Parameters	Value ± Standard deviation
Temperature (°C)	29.00 ± 1.00
Volume of reactor (l)	2.30
Volume of influent (l d^{-1})	14.30 ± 1.60
Concentration of influent (mg CODt l^{-1})	320.00 ± 58.00
B_v (g COD l^{-1} d^{-1})	1.99 ± 0.22
HRT (h)	3.90 ± 0.40
V_{up} (m h^{-1})	1.28 ± 0.12

Note: B_v = Volumetric loading rate; HRT = Hydraulic retention time; V_{up} = Upflow velocity.

Table 2. Characteristics of the raw domestic wastewater fed to the UASB reactor

Parameter		Value ± Standard deviation
pH		7.7 ± 0.2
Alkalinity	(mg $CaCO_3$ l^{-1})	412 ± 45
CODt	(mg l^{-1})	320 ± 58
CODs	(mg l^{-1})	140 ± 35
SS	(mg l^{-1})	165 ± 41
VSS	(mg l^{-1})	132 ± 22
TKN	(mg l^{-1})	33 ± 12
NH^{+}_4-N	(mg l^{-1})	23 ± 9
Ptotal	(mg l^{-1})	10 ± 1
PO_4^{3-}-P	(mg l^{-1})	5 ± 2
SO^{2-}_4-S	(mg l^{-1})	15 ± 2
VFA	(mg l^{-1})	11 ± 3

Note: CODs = Soluble COD

Analytical techniques

Physicochemical parameters were determined in accordance with standard methods (APHA, 1992). COD, SS, VFA and pH were determined 2 to 3 times per week. The pH was measured with a digital pH-meter (Knick-Elscolab n.v., Kruibeke, Belgium). Other parameters mentioned in Table 2 were determined once per week or per two weeks. CH_4 and CO_2 in the biogas were analysed with an Intersmat IGC 120 MB gas chromatograph connected to a Hewlett-Packard 3390 A integrator. S^{2-} was determined by the iodometric method (Fresenius *et al.*, 1988).

Microbial metabolic activity measurement

The maximum specific methanogenic activity (SMA) of the biomass grown in the UASB reactor was measured once every 6 weeks except for the last measurement, which was carried out 4 weeks after the previous one. In total four activities were determined during the experiment. Each of these activities was determined as the

maximum slope of the graph of product formation (g COD-CH_4) per gram of volatile suspended solids (g VSS) against time (d) (Thaveesri *et al.*, 1995b). The SMA test was done in batch reactors with grab sample of biomass. The test was carried out by the pressure-bottle technique using acetate as substrate in an anaerobic dilution medium (Valcke & Verstraete, 1983).

Calculations

All the removal efficiencies were calculated using the formula:

$$\%\ \text{Removal} = 100 \times \left(\frac{C_{influent} - C_{effluent}}{C_{influent}} \right)$$

where C represents the concentration and the subscript indicates whether it is the influent or effluent concentration.

The cumulative sum (CUSUM) method was used to assess the evolution of pH in the influent and effluent and to check whether this evolution changed significantly in time. The CUSUM for each data was calculated as follows (Edeline, 1997b):

$$\text{CUSUM} = \sum_{i=1}^{j} X_i - (J \times M)$$

where X_i, J and M represents respectively the successive pH measured, the serial number of the data X_i, the mean of a certain amount of data taken chronologically from the former. $X_i = f(t)$ is stable when CUSUM is equal or close to zero. The progression of the variances gives an indication of the evolution (abrupt or gradual) of the parameter X_i. In addition, the least significant difference (LSD) test was used to compare the average pH of the different evolution periods indicated by the CUSUM. The test was done with standard software (SPSS for Windows) at a 0.05 level of significance.

RESULTS

COD and SS removal efficiencies of the reactor are presented in Figure 1. The evolution of the efficiencies was characterised by two periods. Period 1, from day

1 to day 40, was marked by an increase of the efficiencies coupled to some minor fluctuations. Period 2, from day 41 to day 154, showed a slight increase of CODt and SS removal efficiencies with an average of 70% and 81%. However, the removal of CODs showed a more pronounced increase since it improved in time, from 32% to 63%. The average removal efficiencies obtained during the 22 weeks of operation were for CODt 65%, for CODs 43%, and for SS 76%.

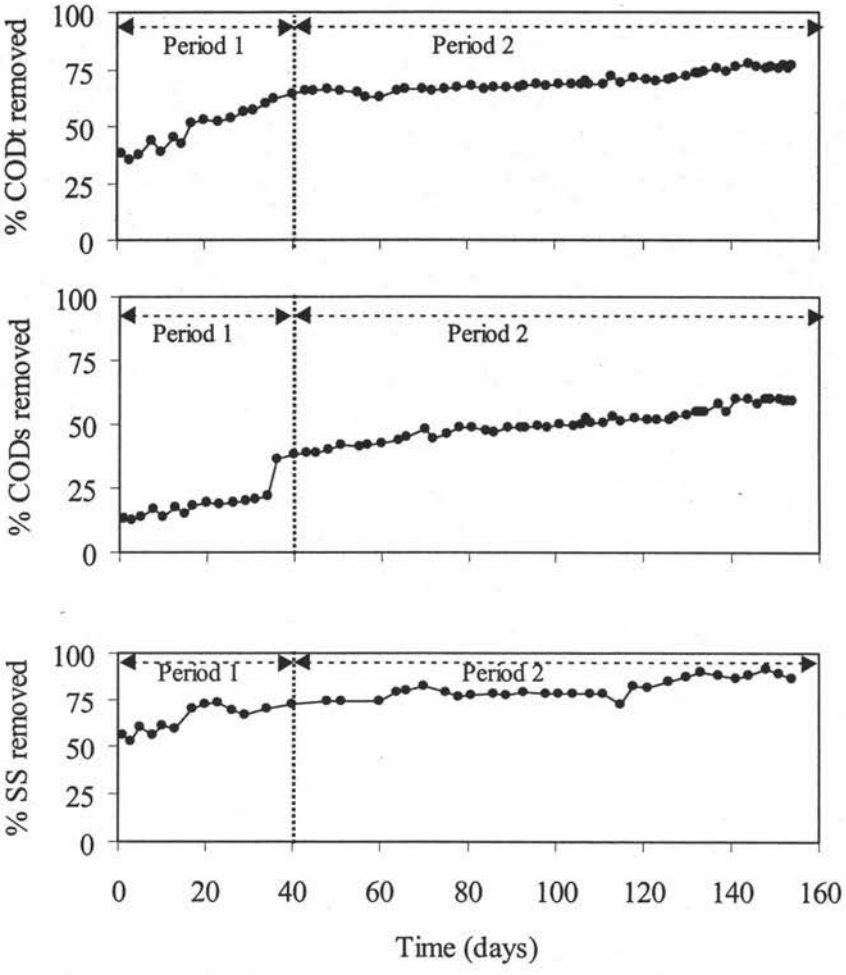


Figure 1. CODt, CODs and SS removal efficiencies as a function of time

A regular measurement of the pH of the feed and the effluent showed the evolution in Figure 2a. From day 1 to day 54 the pH of the effluent was not different from that of the feed. Moreover no biogas was produced, yet the reactor removed up to 65% of CODt and 73% of SS. From day 55 to day 94 the pH of the effluent constantly dropped indicating that acid producing metabolic reactions were occurring. There was, however, no excessive acidification in the reactor since the pH never dropped below 6.8. Although release of biogas from the reactor was not detectable in the gas column connected to the top of the reactor, bubbles of gas moving the sludge bed to the liquid phase were visible. From day 95 to the end of the experiment (154 days) the pH of the effluent increased and was even slightly higher than the pH of the influent.

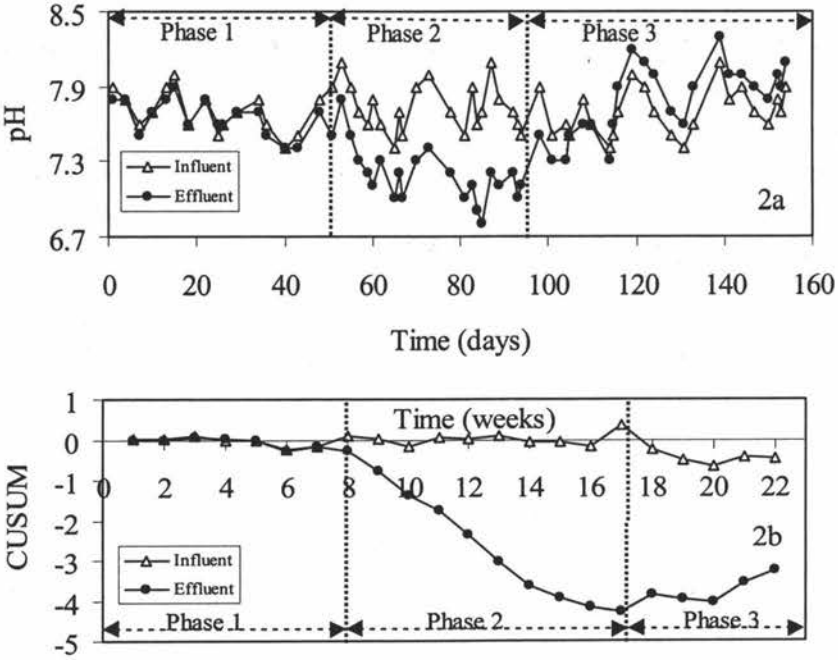


Figure 2. pH (2a) and CUSUM (2b) evolution as a function of time

Figure 2b, which represents the CUSUM of pH gives an excellent overview of the progressive change of the evolution of this parameter as measured in the effluent compared to that of the influent. Three distinct phases were observed, as mentioned above. The results of the statistical test of mean comparison by the LSD method (Table 3) indicated that the average pH during the 3 phases were different from one another. In addition, phase 2 of the influent was statistically different from phase 2 of the effluent.

From day 50 to day 95 the total VFA concentration in the effluent increased slightly (Figure 3). The VFA concentration then decreased gradually and was lower than the detection limit from day 120.

S^{2-} was measured in the effluent. It was mainly detected when phase 3 (from day 104) started and the average concentration over that period was 11.5 mg l^{-1}.

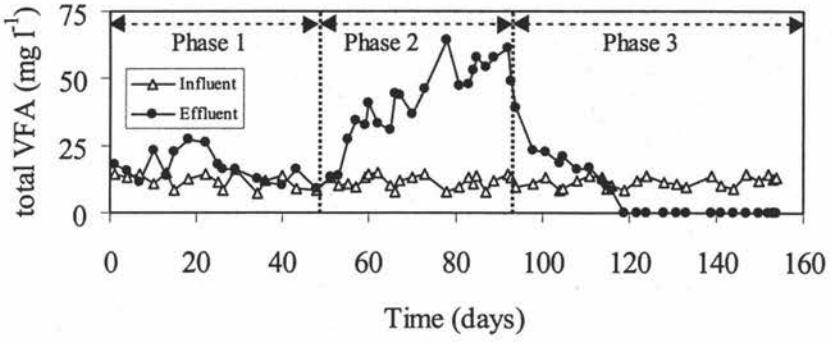


Figure 3. Total VFA evolution in the influent and the effluent as function of time

Table 3. Statistical comparison of the average pH of phases 1, 2 and 3 by the LSD method (each mean of phases in column A is compared with each mean of phases in column B)

A	n	M_A	B	n	M_B	$\lvert M_A - M_B \rvert$	Significant at the 0.05 level
			EP_2	20	7.1450	0.5106	0.000 (yes)
EP_1	18	7.6556	EP_3	23	7.7870	0.1314	0.044 (yes)
			IP_1	18	7.7333	0.0778	0.258 (no)
			EP_3	23	7.7870	0.6420	0.000 (yes)
EP_2	20	7.1450					
			IP_2	20	7.7100	0.5650	0.000 (yes)
EP_3	23	7.7870	IP_3	23	7.7000	0.0870	0.153 (no)

Note: EP_i = Effluent phase i; IP_i = Influent phase i; n = Number of data; M_A = Mean of phases in column A; M_B = Mean of phases in column B; yes = The mean difference is significant at the 0.05 level; no = The mean difference is not significant at the 0.05 level.

The recovery of biogas in the gas column started from day 104 (Figure 4). The first month, biogas was slightly dominated by CO_2. A proportion (%CO_2) / (%CH_4) of 51 / 49 was observed. The proportion of CH_4 gradually increased in time to reach the average proportion of 70%. The total biogas produced corresponded to 70 ml g^{-1}CODt added. It was equivalent to 107 ml g^{-1}CODt removed, which was about 5 times lower than the theoretical value of 500 ml g^{-1} CODt removed. On the basis of this theoretical value it appeared that only 21% of the CODt removed by the reactor were converted into biogas.

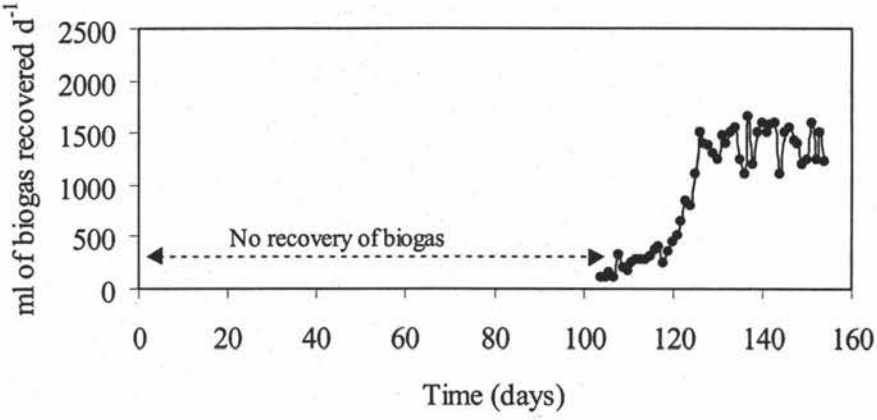


Figure 4. Amount of biogas recovered daily, from the UASB reactor, as a function of time

The specific metabolic activity measurements of the biomass increased from 0.01 (day 42) to 0.1 g COD-CH_4 g^{-1} VSS d^{-1} (day 154). In day 84 and 126 the activity was 0.02 and 0.06 respectively. The increase of the activity corresponded with the increase of the amount of biogas recovered. The concentration of SS and VSS of the reactor sludge blanket increased in time (Table 4). The VSS / SS ratio was almost constant from day 7 to day 42. A significant decrease of the VSS / SS was noticed in day 84 indicating that hydrolysis was indeed occurring as shown by the drop of pH from day 55 to day 94.

Table 4. SS, VSS concentrations and VSS / SS ratio of the reactor sludge blanket

	Days				
	7	42	84	126	154
SS ($g\ l^{-1}$)	3.6 ± 0.2	25.6 ± 0.3	57.1 ± 0.3	92.2 ± 0.5	115.6 ± 0.3
VSS ($g\ l^{-1}$)	2.9 ± 0.4	20.2 ± 0.2	42.4 ± 0.3	65.1 ± 0.2	74.8 ± 0.4
VSS / SS ratio (%)	81	79	74	71	65

DISCUSSION

The reactor dynamics showed three phases. These three phases originated from different processes as indicated by statistical comparison. The first phase characterised by a quasi invariability of the effluent pH and the absence of biogas production presumed that there was a low biochemical activity during this period. This assumption was confirmed by the constant VSS / SS ratio and the low methanogenic activity observed from the 1st to the 6th week of operation. Indeed, active anaerobic sludge has a methanogenic activity comprised between at least 0.08 and 0.2 g CH_4-COD g^{-1} VSS d^{-1} (Lettinga *et al.*, 1980). It appears therefore that the most evident explanation of COD and SS removal during this period was interception in the reactor. It has been frequently reported that a UASB reactor can perform a good removal of organic matter without an effective production of biogas because poorly biodegradable SS is intercepted into the sludge bed (Kalogo & Verstraete, 1999).

The second and third phases of the reactor dynamics were reflected by biochemical reactions. The drop of pH and concomitant increase of VFA concentration in the effluent and also production of gas bubbles are characteristic features of acidogenesis. No biogas was however recovered during the second phase. This was contrary to the removal efficiency of the CODs from day 40 to day 100 and therefore suggested that biogas produced at that time was mainly dissolved in the effluent. Part of biogas could also be lost via the top of the reactor because it was some times opened for pH control. The examination of the system mass balance of the total duration of the experiment indicated that there was a loss of COD of 2% of the total load fed to the reactor (Figure 5). The biogas equivalent to this amount of COD could indeed remain dissolved or lost via the top of the reactor during the second phase.

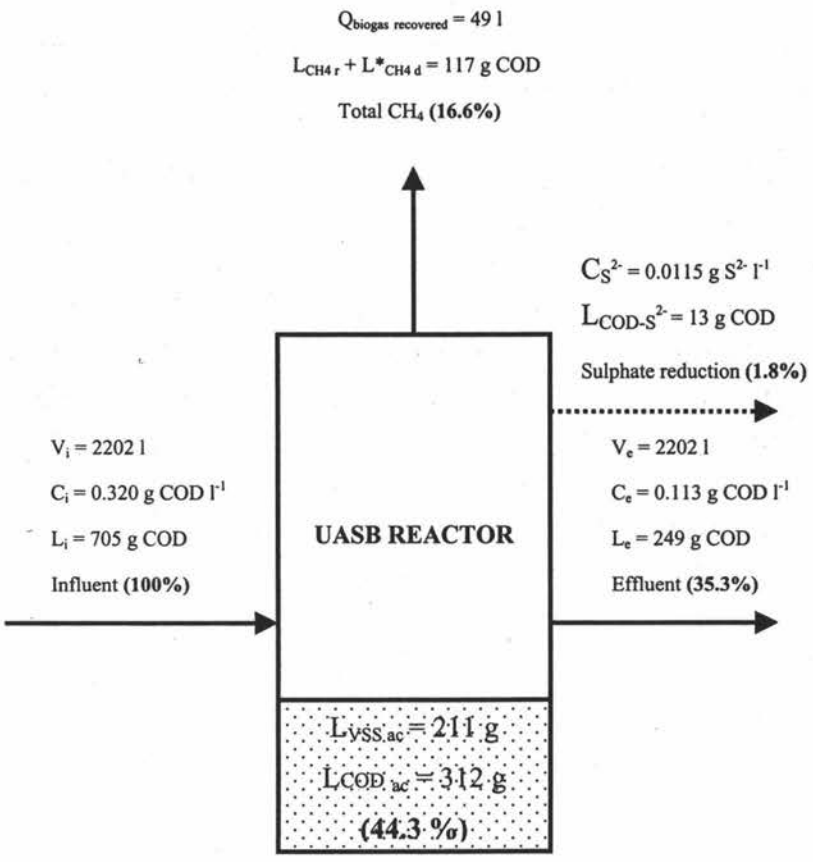


Figure 5. Mass balance of the reactor during the whole experiment

The percentage between brackets are related to the load of COD fed to the reactor. V = Volume; C = Concentration; L = Load; $L_{VSS\ ac}$ = Load of VSS accumulated; $L_{COD\ ac} = L_{VSS\ ac} \times 1.48$; $L_{CH4\ r} = Q_{biogas} \times \%CH_4 \times fc$; fc = 0.350 l CH_4 g^{-1} COD; $L_{CH4\ r}$ = Load CH_4 recovered; $L^*_{CH4\ d}$ = Load CH_4 dissolved; * = Dissolved CH_4 (for the last 50 days) was calculated by multiplying the CH_4 content of the biogas (partial pressure) by the solubility of CH_4 at atmospheric pressure.

Since the mechanism of anaerobic digestion consists of complex substrate hydrolysis and acidification prior to conversion into biogas and the fact that phase 2 was directly followed by methanogenesis, as indicated by effective biogas recovery in phase 3 it is postulated that the second phase corresponded mainly to acidogenesis. This acidogenesis was however not detrimental for methanogenesis to start-up because the amount of VFA remained low. This low amount of VFA was probably due to the relatively short HRT (4 hours) and fair loading rate (1.99 g COD l^{-1} d^{-1}) applied during the experiment. Dinopoulou *et al.* (1988) reported that during the treatment of complex substrate, an increase of HRT from 6 to 8 hours increased the acidification while an increase of the loading rate drastically decreased acidification. It is therefore important that monitoring and control of self-inoculated reactors is focused on this acidification aspect since it can inhibit acidogenesis itself (Aiba *et al.*, 1968) and consequently methanogenesis (Eng *et al.*, 1986).

Although the reactor dynamics showed three phases, the performance (COD and SS removal) could be distinguished in two periods. This seems to indicate that the reactor performance was not directly linked to the occurrence of phase 2 and phase 3. We believe that interception of organic matter was still taking place and was even important as the reactor was operating and the sludge bed was growing. This phenomenon is typical for "manually" inoculated UASB reactors treating domestic wastewater and it is caused by interception of organic compounds in the sludge bed (Van Haandel & Lettinga, 1994).

The average performances of the reactor (biogas recovered, CODt and SS removal) for the whole experiment are comparable with most of those reported elsewhere (Table 5) despite the great variability in HRT and influent concentration between the different cases.

Table 5. Self-inoculated UASB reactors operated with raw domestic wastewater

T	HRT	Influent		B_v	(%) Removal		Biogas production			Start-up (months)	Authors
(°C)	(h)	CODt (mg l^{-1})	SS	(g CODt $l^{-1}d^{-1}$)	CODt	SS	ml gCODta	gCOD-biogas[a] gCODtr	ml gCODtr		
19-23	24-40	460	(+)	(+)	60-75	(+)	115	0.35	(+)	(+)	Grin *et al.* (1983)
19-28	4	627	376	(+)	74	72	80	0.22	(+)	4	Barbosa & Sant'Anna (1989)
20-30	6	563	418	(+)	74	75	(+)	0.1-0.2	50-100	(+)	Draaijer *et al.* (1991)
24-26	5-6	200-300	(+)	(+)	55-75	67-81	(+)		190[b]	3	Lettinga *et al.* (1993)
30	24-3	600	(+)	0.4-6	90	(+)	(+)		(+)	(+)	Gnanadipathy & Polprasert (1993)
(+)	5.2	(+)	(+)	(+)	50-60	(+)	(+)		(+)	5	Schellinkhout & Osorio (1994)
18-31	2.5	924	118	(+)	18	75	(+)		(+)	(+)	Wang (1994)
(+)	8	(+)	(+)	5.625	24-50	29-73	(+)		(+)	4	Tare *et al.* (1997)
(+)	8	(+)	(+)	1.10	49-65	50-76	(+)		(+)	4	Tare *et al.* (1997)
20-30	6	500	418	2.08	80	85	(+)		(+)	4	Tare *et al.* (1997)
10-26	4	470	(+)	1-1.7	60	70	(+)		(+)	(+)	García *et al.* (1998)
29	4	320	165	1.99	65	76	70	0.21	107	1.5[c] ; 3.5[d]	This work

Note: (+) = Not indicated; CODta = CODt added; CODtr = CODt removed; T = Temperature; B_v = Volumetric loading rate; [a] = Value calculated; [b] = ml CH_4 g^{-1} CODt removed; [c] = Period of primary start-up; [d] = Period before secondary start-up.

HRT and influent concentration, however, do not seem to be the main factors of the success of self-inoculation. This success is rather dependent on the septic character (concentration of micro-organisms) and the fraction of particulate COD of the wastewater (Lettinga *et al.*, 1993). Comparing the fraction of particulate COD of 35% and 76% previously observed by Grin *et al.* (1983) and Barbosa & Sant'Anna (1989) respectively with the 56% of the wastewater used in this work, it can be concluded that the feed contained a substantial fraction of particulate COD.

The results of this work confirmed that biogas recovery during self-inoculation is low compared to what should be expected according to the removal efficiency. This suggests that interception of substrate is the most dominant process during the start-up of a self-inoculated UASB reactor. Indeed the COD-biogas recovered / CODt removed ratio observed in this work was only 0.21. Interception represented 68% of the 65% of the CODt removed by the reactor since 3% of COD were removed by sulfate reduction as 11.5 mg $S^{2-}l^{-1}$ were detected in the effluent when phase 3 was started-up. The large discrepancy, between CODt removed and biogas recovered, observed in this work is related to the long time needed (14 weeks) for start-up of effective biological conversion of the intercepted organic compounds into biogas recoverable. It appears therefore that efforts for optimisation of the self-inoculation process have to focus on shortening the time needed to reach an effective biological start-up period probably by improving the retention of microorganisms and / or supplying co-substrates in the reactor.

In view of the results observed in this work, at least two types of start-up period should be considered during the operation of a self-inoculated UASB reactor treating raw domestic wastewater. Because of its particular character, the first phase mentioned above should be considered as primary start-up. Since we believe that interception is not limited in time, the primary start-up should not be regarded as interception alone but more as a period of induction of biochemical

reactions.

The second start-up period should be considered as the beginning of biogas recovery. Biochemical reactions occurring between these two periods should be considered as a lag phase of the second start-up period. In this context the duration of the second start-up period defined here, agrees with the start-up period reported for the most successful self-inoculated UASB reactors as indicated in Table 5. According to the experimental results observed in this work and due to the fact that the biochemical reactions observed were consistent with the mechanism of anaerobic digestion, a basic model describing the dynamics of a self-inoculated UASB reactor treating raw domestic wastewater is proposed as shown in Figure 6.

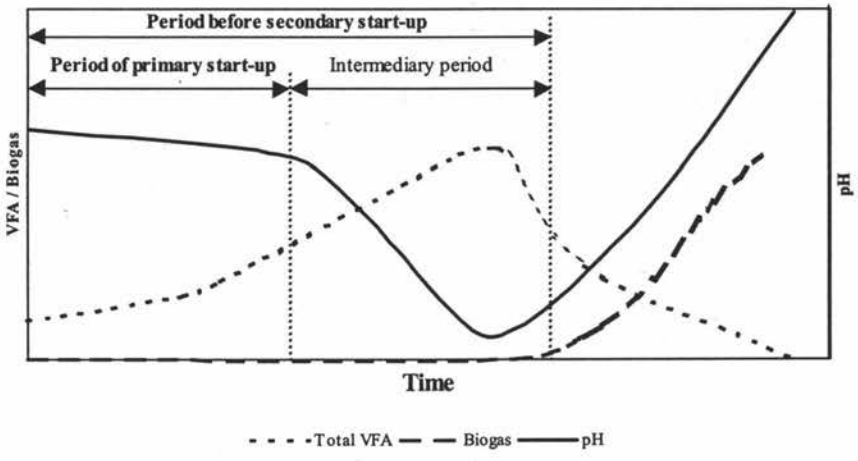


Figure 6. Proposed model to describe the dynamics of a self-inoculated UASB reactor treating raw domestic wastewater: evolution of the total VFA concentration in the effluent, the pH of the effluent and the biogas production (not to scale)

CONCLUSIONS

The start-up of a UASB reactor, without any specific inoculum, treating raw domestic wastewater was studied. After 22 weeks of operation at 29 °C with a HRT of 4 hours, the reactor removed up to 80% of CODt, 60% of CODs and 90% of SS. However 68% of CODt removed was due to its interception by the anaerobic sludge. The results confirm that the operation of a UASB reactor on raw domestic wastewater without inoculation is feasible, yet with slow biological conversion. The long time needed (14 weeks in this work) for start-up of effective biological degradation reflected a large discrepancy between CODt removed and biogas recovered after 22 weeks of operation.

ACKNOWLEDGEMENTS

The author Y. Kalogo would like to thank the Government of his country (Republic of Côte d'Ivoire) for the financial support through a grant. The authors express their gratitude to G. Rombaut and F. Hammes for statistical analysis and critical comments.

CHAPTER III

START-UP OF A SELF-INOCULATED UASB REACTOR TREATING DOMESTIC WASTEWATER - PART II. PROCESS OPTIMISATION

Kalogo Y. & Verstraete W.

A modified version of this text was accepted for publication as:

Kalogo Y. Séka M.A. & Verstraete W. (2000) Enhancing the start-up of a UASB reactor treating domestic wastewater by adding a water extract of *Moringa oleifera* seeds. *Applied Microbiology and Biotechnology* (in press)

START-UP OF A SELF-INOCULATED UASB REACTOR TREATING DOMESTIC WASTEWATER - PART II. PROCESS OPTIMISATION

Abstract - Water extract of *Moringa oleifera* seeds (WEMOS) was used to enhance the start-up of a self-inoculated upflow anaerobic sludge blanket (UASB) reactor treating raw domestic wastewater. Two reactors labelled control (RC) and WEMOS addition (RW) were started without any special inoculum. Both reactors were fed continuously for 22 weeks with domestic wastewater containing an average total chemical oxygen demand (CODt) of 320 mg O_2 l^{-1} and suspended solid (SS) of 165 mg l^{-1}. The reactors were operated during the entire experimental period at 29 °C and at a hydraulic retention time (HRT) of 4 hours. The RW reactor received 2 ml of WEMOS per litre influent. The WEMOS solution was prepared on the basis of a 2.5% (w/v) of grounded *M. oleifera* seeds in water.

The results of 22 weeks operation showed an improvement of the performance of RW compared to that of RC. The addition of WEMOS into the feed (a) shortened the biological start-up period by 20%, (b) increased the acidogenic and methanogenic activity by a factor of 2.4 and 2.2 respectively, (c) increased the specific biogas production by a factor 1.6, (d) allowed the aggregation of coccoid bacteria and growth of microbial nuclei, precursors of anaerobic granulation.

Key words: Anaerobic digestion, domestic wastewater, *M. oleifera*, UASB reactor, self-inoculation.

INTRODUCTION

Inoculation of an upflow anaerobic sludge blanket (UASB) reactor by granular sludge can be a costly operation. Substrates such as raw domestic wastewater contain both anaerobic and facultative anaerobic microorganisms (Bitton, 1999; Crowther & Harkness, 1975) and it may therefore appear economical to start the

reactor without any specific inoculation. To date, different investigations on self-inoculation of UASB reactors treating domestic wastewater have been reported. Barbosa & Sant'Anna (1989) and Grin *et al.* (1983) reported that the process was feasible. The former even obtained granules up to 8 mm diameter. These results motivated the start-up of full-scale installations treating raw domestic wastewater without inoculation (Draaijer *et al.*, 1992; Lettinga *et al.*, 1993; Schellinkhout & Osorio, 1994; Tare *et al.*, 1997). However, the chemical oxygen demand (COD) removal efficiencies of these installations were characterised by a great variability since they ranged between 24 and 80%. Moreover, for none of them was granular growth reported. García *et al.* (1998), Gnanadipathy & Polprasert (1993), and Wang (1994) reported unsatisfactory self-inoculation of UASB reactors on domestic wastewater, which was ascribed to weak retention and low activity of biomass in the reactors. Overall, it appears that thus far self-inoculation of a UASB reactor has been unreliable.

In recent years it has been demonstrated that supplementation of anaerobic reactors with some natural polymers enhanced their start-up (El-Mamouni *et al.*, 1998). The present work investigated the enhancement of start-up of self-inoculation by adding a water extract of *Moringa oleifera* seeds (WEMOS). *M. oleifera* is a tropical plant belonging to the family *Moringaceae*. The WEMOS is effective in flocculating organic matter and removing microorganisms from raw water (Folkard & Sutherland, 1994; Olsen, 1987; Schultz & Okun, 1984). It is currently used to treat drinking water where it has shown its efficiency as a coagulating agent (Muyibi & Evison, 1995; N'Dabigengesere & Narasiah, 1998a). The active agents of the WEMOS are dimeric cationic proteins having a molecular weight of 13 kDa and an isoelectric point between 10 and 11 (N'Dabigengesere *et al.*, 1995). The amino acid sequences of the most important protein have been determined by Gassenschmidt *et al.* (1995). The WEMOS solution contains several types of carbon sources, e.g. carbohydrates, lipids, lignin etc and also nutrients, e.g. N, P, metal ions etc which are capable of supporting microbial growth. It was therefore hypothesised that a continuous

supply of WEMOS to a UASB reactor treating raw domestic wastewater would result in enhancing its start-up period.

MATERIALS AND METHODS

M. oleifera seeds and their preparation

The seeds of *M. oleifera* were obtained from the Centre National de Semences Forestières in Burkina Faso (West Africa). The dry pods of the *M. oleifera* were harvested in Fada (Burkina Faso) in March 1993. Before using, the barks enveloping the seeds were removed and the seeds were subsequently grounded to powder in a porcelain mortar. Recently, the extraction of the coagulation active agents of *M. oleifera* was improved by using a sodium chloride (NaCl) solution (Okuda *et al.*, 1999). This technique, however, removes carbohydrates available in the seeds. In order to maintain the entire nutrient-potential available in the seeds, extraction in the present work was done according to the method previously described by N'Dabigengesere *et al.* (1995). The *M. oleifera* solution was prepared by adding 2.5 g of the powder to 100 ml distilled water. The mixture was centrifuged (30 min; 10 000 rpm), and the resulting supernatant was filtered (8 µm). The composition of the filtrate (WEMOS) analysed according to standard methods (APHA, 1992) is indicated in Table 1. Glucose was measured according to the glucose oxidase reaction using a Biochemistry Analyser (Model 2500 Select, Biorad). A fresh solution of WEMOS was prepared weekly and stored at 4 °C. During the extraction of the active agent of WEMOS, only a part of the dry matter is dissolved. This is estimated to about 25% of the total dry matter (N'Dabigengesere *et al.*, 1995). The dose of WEMOS is therefore expressed in ml l^{-1} instead of mg l^{-1}.

Batch experiments

A series of preliminary physico-chemical studies with a jar test (Geppert, type MSP6, Rührtechnik, Germany) were carried out. The objectives of those studies were to determine (1) a working concentration of WEMOS, (2) the effect of WEMOS on bacterial removal, and (3) the effect of pH variation of the water being treated on the performance of WEMOS. The first investigation was carried out with the following concentrations of WEMOS: 0; 2; 8 and 16 ml l^{-1}. COD and SS removal were the main parameters used to evaluate the effect of the different concentrations tested. In the second investigation the influence of the same concentrations of WEMOS on bacterial removal was studied. Total coliform count (TC) was taken as parameter to evaluate the effect of each dose. TC was determined in the supernatant fluid and wasted sludge. In the last investigation, the pH of the raw wastewater was fixed successively from 4 to 9.5 using HCl (Vel, Leuven, Belgium) or NaOH (Vel, Leuven, Belgium). SS removal was used to evaluate the efficiency of WEMOS (working dosage) at the different pH's.

Operation of the UASB reactors

Two identical UASB reactors (2.3 l volume) labelled control reactor (RC) and WEMOS addition (RW), were set up and operated using the parameters summarised in Table 2. The reactors were made of glass (Schott-Duran, Germany) with a height of 90 cm and an internal diameter of 5 cm. On top, a decantation sphere of a 0.5 l volume was fitted. The reactors were fed by using peristaltic pumps (Watson Marlow, 313S, Germany). The reactors were operated without specific inoculation and fed continuously for 22 weeks with raw domestic wastewater (Table 3), which was obtained from the Ossemeersen domestic wastewater treatment plant (Ghent, Belgium). The volatile fatty acid (VFA) content of the feed was ca 4% of the total COD (CODt). The COD in the form of SS was 56% of the CODt. In the case of the RW reactor, 2 ml of

WEMOS was dosed per litre wastewater. These additions were made once per day directly to the reactor feed in order to guarantee an optimal effect (Folkard & Sutherland, 1994).

Table 1. Nutrient and organic matter content of the WEMOS (extract of 2.5 g powder per 100 ml water)

Parameter		Value ± Standard deviation
pH		5.74 ± 0.02
Alkalinity	(mg $CaCO_3$ l^{-1})	52.45 ± 1.34
TKN	(mg l^{-1})	684.85 ± 0.49
NH_4^+-N	(mg l^{-1})	2.49 ± 0.23
NO_3^--N	(mg l^{-1})	11.56 ± 1.44
PO_4^{3-}-P	(mg l^{-1})	102.11 ± 3.15
Al	(mg l-1)	0.41 ± 0.01
Cu	(mg l-1)	0.38 ± 0.18
Fe	(mg l-1)	3.63 ± 1.59
Mo	(mg l^{-1})	35.00 ± 4.00
Ni	(mg l^{-1})	0.29 ± 0.08
Zn	(mg l^{-1})	1.48 ± 0.39
Co	(mg l^{-1})	0.05 ± 0.01
Ca	(mg l^{-1})	375.52 ± 0.71
K	(mg l^{-1})	78.91 ± 4.38
Mg	(mg l^{-1})	26.25 ± 1.77
Na	(mg l^{-1})	11.12 ± 1.41
COD	(g l^{-1})	25.00 ± 3.00
Glucose	(mg l^{-1})	925.00 ± 3.00

The top of each reactor was connected to a gas column, while the pH of the liquid in these gas columns was lowered to 4, by addition of HCl, in order to avoid the dissolution of CO_2 of the biogas. Methyl orange (Vel, Leuven, Belgium) was added to the liquid as indicator of the biogas level in the columns. Figure 1 shows the schematic representation of the experimental set-up used during the study .

Table 2. Operating parameters of the UASB reactors

Parameters	Value ± Standard deviation
Temperature (°C)	29.00 ± 1.00
Volume of reactor (l)	2.30
Volume of influent (l d^{-1})	14.30 ± 1.60
Concentration of influent (mg COD l^{-1})	320.00 ± 58.00
B_v (g COD l^{-1} d^{-1})[a]	1.99 ± 0.22
HRT (h)	3.90 ± 0.40
V_{up} (m h^{-1})	1.28 ± 0.12

Note: [a] Calculated on the basis on the COD present in the raw wastewater, V_{up} = Upflow velocity, B_v = Volumetric loading rate.

Table 3. Characteristics of the raw wastewater fed to the reactors

Parameters		Value ± Standard deviation
pH		7.7 ± 0.2
Alkalinity	(mg $CaCO_3$ l^{-1})	412 ± 45
CODt	(mg l^{-1})	320 ± 58
CODs	(mg l^{-1})	140 ± 35
SS	(mg l^{-1})	165 ± 41
VSS	(mg l^{-1})	132 ± 22
TKN	(mg l^{-1})	33 ± 12
NH_4^+-N	(mg l^{-1})	23 ± 9
Ptotal	(mg l^{-1})	10 ± 1
PO_4^{3-}-P	(mg l^{-1})	5 ± 2
SO_4-S	(mg l^{-1})	15 ± 2
VFA	(mg l^{-1})	11 ± 3
Al	(mg l^{-1})	ND
Cu	(mg l^{-1})	<0.05
Fe	(mg l^{-1})	2.3 ± 0.6
Mo	(mg l^{-1})	ND
Ni	(mg l^{-1})	0.05 ± 0.02
Zn	(mg l^{-1})	0.41 ± 0.09
Co	(mg l^{-1})	<0.1
Ca	(mg l^{-1})	75.00 ± 7.00
K	(mg l^{-1})	11.88 ± 1.94
Mg	(mg l^{-1})	10.00 ± 1.00
Na	(mg l^{-1})	102.50 ± 3.50

Note: ND = Not detected.

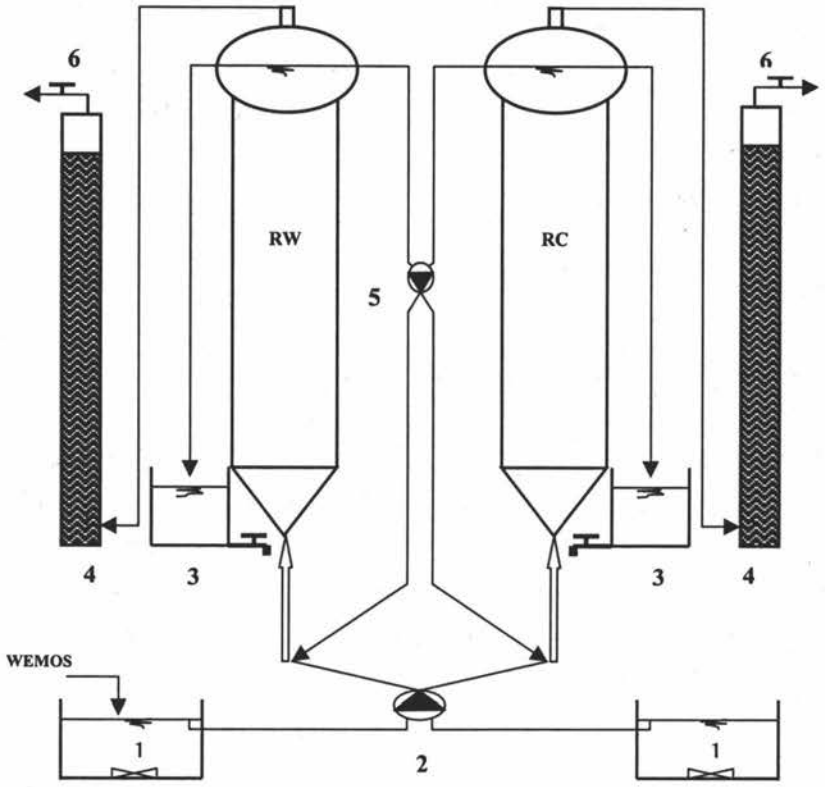


Figure 1. Schematic representation of the experimental set-up (not to scale)

1. Influent tanks (raw domestic wastewater) with mixers; **2**. Feeding pump; **3**. Effluent tanks;. **4**. Biogas column filled with water at pH < 4; **5**. Recycling pump; **6**. Biogas sampling point; **RC**. Control reactor without biomass; **RW**. WEMOS addition reactor without biomass.

Analytical techniques

Physico-chemical parameters were determined in accordance with standard methods (APHA, 1992). COD, SS, VFA and pH were determined 2 to 3 times per week, while the other parameters mentioned in Table 3 were determined once per week or per two weeks. The pH was measured with a digital pH-meter

(Knick-Elscolab n.v., Kruibeke, Belgium). TC were enumerated by plating count techniques as described by Kersters *et al.* (1995). CH_4 and CO_2 in the biogas were analysed with a gas chromatograph (Intersmat IGC 120 MB) connected to a Hewlett-Packard 3390 A integrator. S^{2-} was determined by the iodometric method (Fresenius *et al.*, 1988).

Microbiological examination of the biomass

The activity of the biomass grown in UASB the reactors was measured in triplicate at the end of the experiment. The specific activity was determined as the maximum slope of the graph of product formation (g COD-CH_4 or meq H^+) per gram of volatile suspended solids (VSS) against time (Thaveesri *et al.*, 1995b). The specific acidogenic activity (SAA) was measured according to the technique described by Vanderhaegen *et al.* (1992), using dextrose as substrate. The maximum specific methanogenic activity (SMA) test was carried out by the pressure-bottle technique using acetate as substrate in an anaerobic dilution medium (Valcke & Verstraete, 1983). The activities were determined in batch reactors with grab sample of biomass.

The microbial morphology was examined by means of scanning electron microscopy (SEM), apparatus Jeol JSM 840, after fixation of sample sludge with glutaraldehyde (Sigma, St. Louis, USA) solution (Zellner *et al.*, 1991). The sludge samples were examined with a working distance (WD) of 29 mm from the surface of the samples.

Extracellular polysaccharides (EPS) were quantified by spectrophotometer (Uvikon, Kontron instruments, Italy) at an optical density of 520 nm with the uronic acid determination of Blumenkrantz & Asboe-Hansen (1973) using glucuronic acid as standard. Prior to this determination, the sludge samples were treated as described by De Beer *et al.* (1996).

Methanosarcina species were determined using *Methanosarcina* medium described by Atlas (1993) with methanol as substrate. *Methanosaeta* species

were enumerated according to the same procedure with 15 mM acetate as substrate (Wu *et al.*, 1992). Both methanogen species were enumerated after incubation at 37 °C over a period of two months. Under the conditions of incubation both species had ample time for growth. The point was to observe the relative preponderance of both species.

Statistical analysis

The difference between the performance of the reactors was evaluated by using the t-test on paired observations as described by Trousset & Morin (1994). The test was performed with standard software (SPSS for Windows) at a 0.05 level of significance.

RESULTS

Batch experiments

Table 4 shows the results of the effect of the dose of WEMOS on COD and SS removal. Doses lower than 2 ml did not show an effective flocculation of the organic matter. Higher doses showed better flocculation but released an excessive COD compared to the COD present in the wastewater. The effect of different doses on the removal of TC from the supernatant fluid showed a decrease of the concentration of the bacteria with the increase of the dose of WEMOS (Figure 2). TC concentrations in the wasted sludge were determined. The results showed that bacteria removed from the liquid phase were not dead since the bacteria in the sludge were enumerated by plating count. The TC concentrations in the sludge increased with the increase of the dose of WEMOS (Figure 2). This suggested that the bacteria were likely removed concomitantly with the removal of SS.

Figure 3 shows the profile of SS as a function of pH variation. The removal efficiency of SS was not sensitive to pH variation in the range of 4 to 9.5. In

conclusion to these preliminary investigations a working dosage of 2 ml of WEMOS per litre of influent was chosen as supplement for the continuous operation of the RW reactor.

Table 4. Effect of the dose of WEMOS, 2.5% (w/v), on COD and SS removal eff

Dose (ml l^{-1})	SV_{60} (ml l^{-1})	pH	Alkali-nity[a]	CODt (mg l^{-1})	CODs (mg l^{-1})	SS (mg l^{-1})
*	+	7.5 ± 0.1	431 ± 10	362 ± 8	184 ± 9	180 ± 11
0	1.4 ± 0.1	7.4 ± 0.2	420 ± 13	272 ± 11	173 ± 7	126 ± 9
2	1.8 ± 0.2	7.4 ± 0.3	418 ± 6	274 ± 7	181 ± 5	104 ± 6
8	2.6 ± 0.1	7.3 ± 0.1	415 ± 14	280 ± 10	199 ± 10	61 ± 8
16	5.3 ± 0.3	7.4 ± 0.3	415 ± 19	294 ± 15	215 ± 6	58 ± 7

Note: * = Raw wastewater completely mixed; + = Not determined; SV_{60} = Volume of sludge settling after 1 hour, [a] = mg $CaCO_3$ l^{-1}; 0 ml l^{-1} is relative to the supernatant fluid obtained from natural sedimentation.

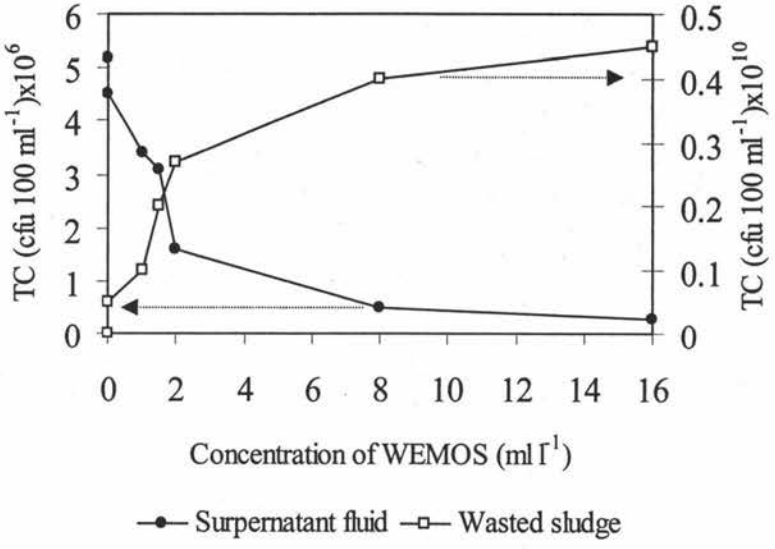


Figure 2. TC concentrations in the supernatant fluid and the wasted sludge as a function of the concentration of WEMOS

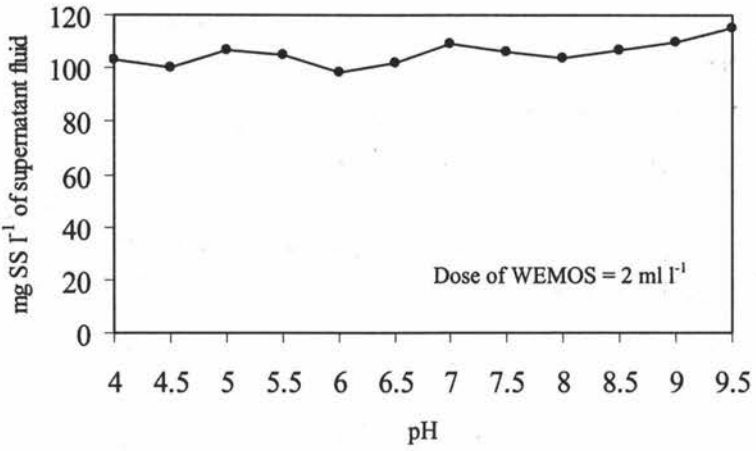


Figure 3. SS concentrations in the supernatant fluid as function of pH of the raw wastewater: SS concentration of the raw wastewater = 180 ± 11 mg l^{-1}.

Continuous experiment

Performance of the reactors

COD and SS removal efficiencies of RC and RW are presented in Figure 4. These efficiencies were calculated based on the COD and SS of the raw wastewater and of the reactor effluents, thus not considering the amount of COD and SS introduced by *Moringa* addition in RW. By this approach, it was assessed whether the WEMOS had any positive effect on the reactor performance. Both reactors showed a similar behaviour during the 22 weeks of experimentation. The evolution of the removal efficiencies, was characterised by two phases. Day 1 to day 40, was marked by an increase of the efficiencies coupled to some minor fluctuations. Day 41 to day 154, showed a slight increase of CODt and SS removal efficiencies with an average of respectively, 73% and 83% for RW and 70% and 81% for RC. However, the removal of CODs showed a more pronounced increase since it improved in time, from 35% to 65% for RW and from 32% to 63% for RC. Overall removal efficiencies obtained were for CODt 68%, for CODs 46%, for SS 78% in RW against respectively 65%, 43%, 76% in

RC. Statistical analysis by t-test on paired observations showed that the removal efficiencies of RW although only slightly higher compared to those of RC, were different at a 0.05 significance level.

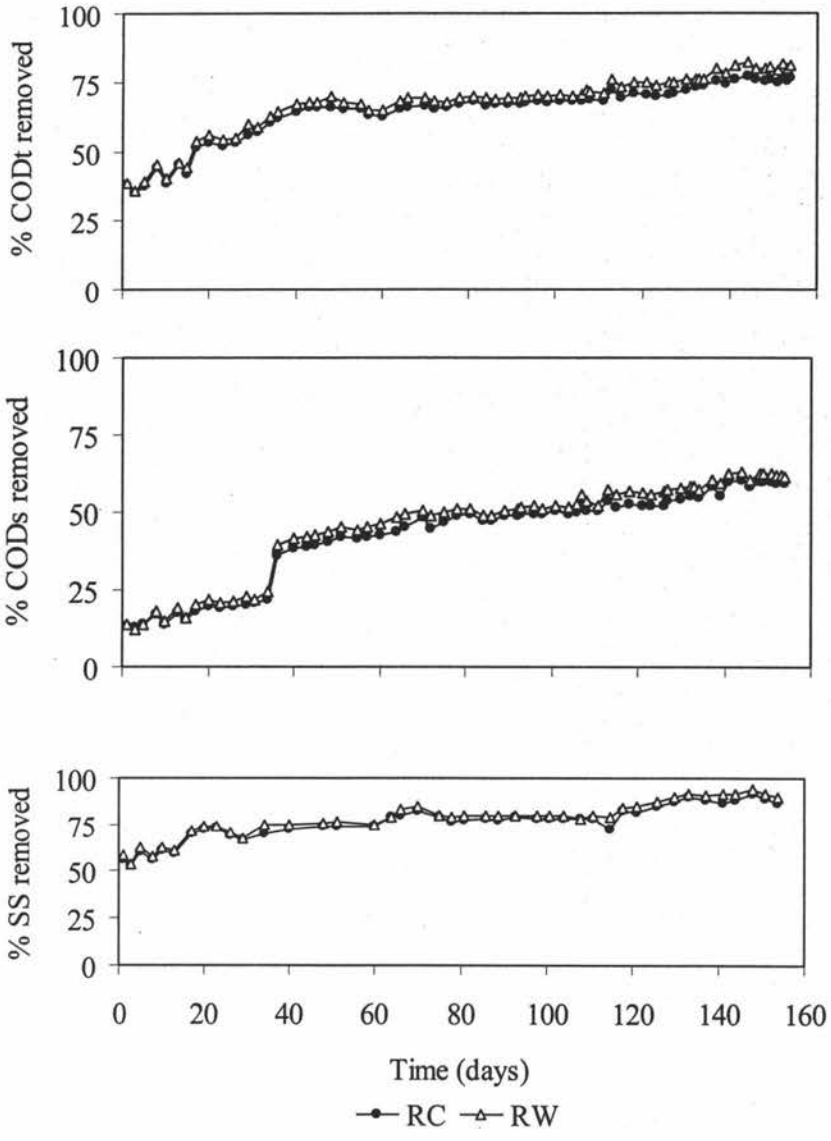


Figure 4. CODt, CODs and SS removal efficiencies of the UASB reactors

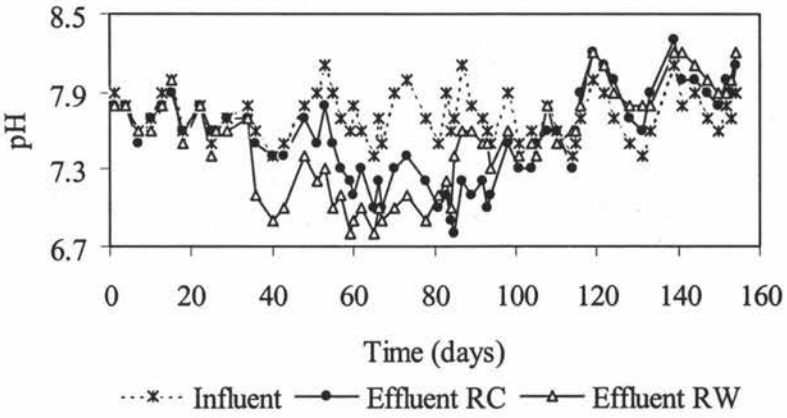


Figure 5. pH evolution in the influent and the effluents of the UASB reactors

The pH of the feed and the effluents was regularly measured during the study (Figure 5). From day 1 to day 34 for RW and day 1 to day 54 for RC the pH of the effluent was not different from that of the feed. But from day 36 to day 84 for RW and day 55 to day 94 for RC the pH of the effluent constantly dropped indicating that acidogenesis was occurring. There was, however, no excessive acidification in the reactors since the pH never dropped below 6.8. From day 85 for RW and day 95 for RC to the end of the experiment (154 days) the pH increased and was even slightly higher than the pH of the influent. The total VFA concentration in the effluent of both reactors increased slightly from day 30 to day 80 for RW and from day 50 to day 95 for RC (Figure 6).

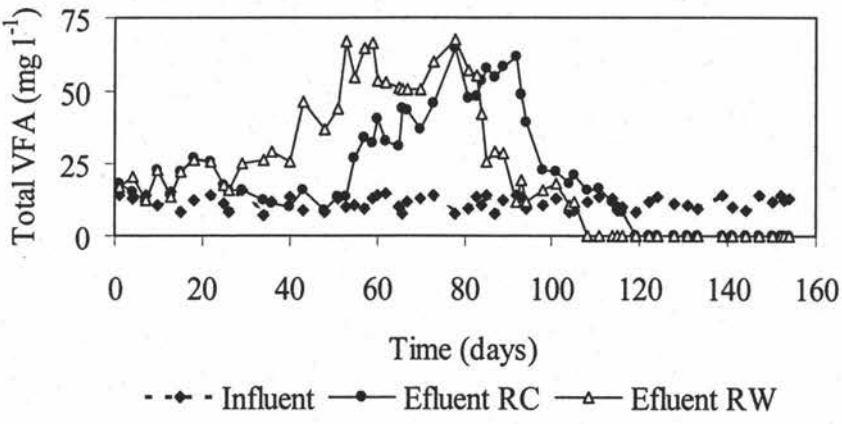


Figure 6. Total VFA concentration in the Influent, the efluent of RC and the efluent of RW

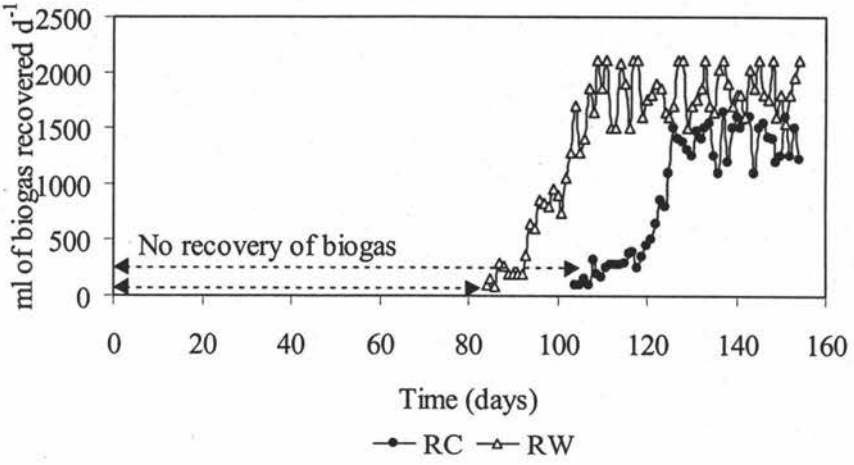


Figure 7. Amount of biogas recovered daily, from the UASB reactors as a function of time

Biogas production (Figure 7) was noticed after 84 days for RW and after 104 days for RC. This corresponded to a shortening of the start-up period by 20%. The increase in CODs removal from day 40 to day 100 without concurrent biogas recovery was surprising. Although biogas was not recovered in the gas columns connected to the top of the reactors, bubbles of gas moving the sludge

bed to the liquid phase were visible. Probably the biogas produced mainly left the reactor dissolved in the effluent. The first month, biogas produced was slightly dominated by CO_2. The proportions $\%CO_2$ / $\%CH_4$ of 53/47 for RW and 51/49 for RC were the consequence of the dominance of acidogenesis over methanogenesis as corroborated by the pH and VFA in the effluents. The proportion of CH_4 gradually increased in time to reach the average proportion of 70/30 for both reactors. The total biogas produced during the overall experiment was 103 l for RW and 49 l for RC. This production corresponded to 126 ml g^{-1} CODt added for RW and 70 ml g^{-1} CODt added for RC. It was also equivalent to 175 ml g^{-1} CODt removed and 107 ml g^{-1} CODt removed respectively.

About 3% of the total COD removed in both reactors went to sulfate reduction since on average 11.5 and 9 mg S^{2-} l^{-1} were respectively detected in the effluent of RC and RW after the start-up of biogas production. This suggests that biological sulphate reduction took place in the reactors, but with limited efficiency.

Microbial metabolic activity measurement and morphology examination

Specific metabolic activity measurements at the end of the experimental period are presented in Table 5. They show that the acidogenic and methanogenic activities of the biomass from the RW reactor were higher than those from the RC reactor (factor 2.4 and 2.2 respectively). The biomass did not differ in EPS content (Table 5). Yet, microbial enumeration revealed a much higher number of *Methanosaeta* in the RW biomass (Table 5).

Table 5. Specific activities, EPS content and Methanogen enumeration of the UASB reactors biomass

	SAA (meq H^+ g^{-1}VSS h^{-1})	SMA (g COD-CH_4 g^{-1}VSS d^{-1})	EPS (µg uronic acid g^{-1}VSS)	*Methano-sarcina* (n ml^{-1})	*Methano-saeta* (n ml^{-1})
RC	0.40 ± 0.05	0.10 ± 0.03	422 ± 38	N.D	$9.1\ 10^3$ $\pm 1.2\ 10^3$
RW	0.95 ± 0.03	0.22 ± 0.04	429 ± 36	N.D	$4.5\ 10^6$ $\pm 1.1\ 10^6$

Note: N.D = Not detected; n = Number of bacteria.

Table 6. Performance of the UASB reactors, biogas yield and mineral composition

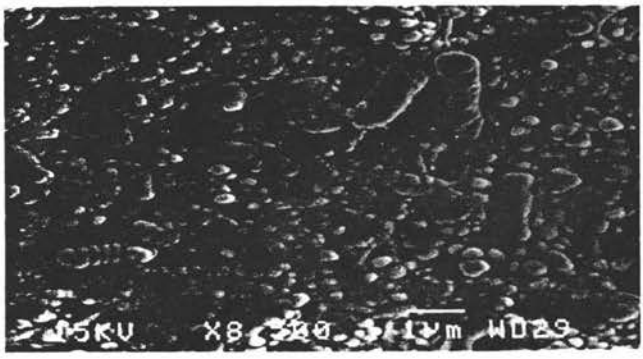
Parameter	RC	RW
Influent CODt for the entire test run (g)	705	815[a]
CODt removed (g)	458	589
Overall efficiency (%)	65	72
Y_{biogas}(ml g^{-1}COD removed)	107	175
$Y_{sulphide}$ ($gS^{2-}g^{-1}$CODremoved)	0.017	0.015
Al (mg g^{-1} VSS)	ND	0.007
Cu (mg g^{-1} VSS)	0.14	0.21
Fe (mg g^{-1} VSS)	4.7	16.0
Mo (mg g^{-1} VSS)	ND	0.37
Ni (mg g^{-1} VSS)	0.18	0.19
Zn (mg g^{-1} VSS)	1.6	2.2
Co (mg g^{-1} VSS)	0.17	0.26
Ca (mg g^{-1} VSS)	245	327
K (mg g^{-1} VSS)	41	63
Mg (mg g^{-1} VSS)	43	44
Na (mg g^{-1} VSS)	157	233

Note: [a] Due to the 50 mg of COD, supplied by the WEMOS, per litre of influent, ND = Not detected.

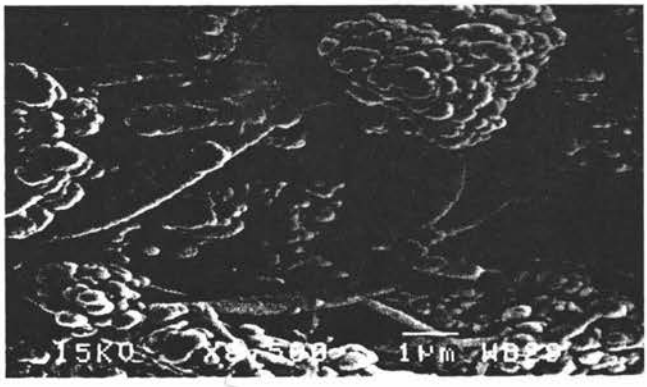
The biomass from the two reactors could also be distinguished by the colour thereof. The biomass from RC was primarily grey while that from RW was primarily black. Analysis of the elemental composition of biomass from both

reactors revealed that the biomass in the RW reactor was more enriched in metal ions than that of the RC reactor (Table 6).

The SEM (Figure 8) showed that coccoid-like bacteria dominated the microbial populations developed in RC. Rod shaped microorganisms were also present. Observation of the biomass from RC, by SEM, showed a microbial community with a dense outlook and free cells. In contrast the biomass from RW displayed aggregates and also filamentous cells, suggesting probably the start-up of granule formation.



Biomass from the control reactor (RC)



Biomass from the WEMOS addition reactor (RW)

Figure 8. Scanning electron microscopy of the biomass from the reactors: WD = working distance between the ocular and the surface of the sluge sample in mm

DISCUSSION

Throughout the experimental period, the RW reactor performed constantly better than the control reactor, which was indicated with statistically significant differences. The better efficiencies of RW are not due to the amount of COD-WEMOS added since this was corrected for in the calculations. The results demonstrate that the WEMOS had a direct positive impact on the RW reactor performance. This is likely due to the strong coagulation and sedimentation effect of the WEMOS (N'Dabigengesere *et al.*, 1995; N'Dabigengesere & Narasiah, 1996; N'Dabigengesere & Narasiah, 1998a), which improved the retention of organic matter in RW.

The amount of biogas produced in the RC (70 ml g^{-1} CODt added) was lower than that reported by Barbosa & Sant'Anna (1989) and Grin *et al.* (1983) for self-inoculated reactors. These authors respectively reported 80 and 115 ml g^{-1} CODt added) for a similar type of wastewater. However, the results of RC were better than those reported by García *et al.* (1998), Gnanadipathy & Polprasert (1993) and Wang (1994). According to Lettinga *et al.* (1993) the success of self-inoculation of an UASB reactor is dependent on the septic character (concentration of microorganisms) of the feed. This probably explains the modest results of RC, since raw material collected from domestic septic tanks was regularly discharged in the sewerage system where the feed used in this study was collected from. However, the low biogas production of RC compared to RW suggests that the physico-chemical removal of the substrate by the former was not followed by adequate biological conversion into biogas of the retained organics.

The methanogenic activity of RW (0.22 g COD-CH_4 g^{-1}VSS d^{-1}) was higher than the values of 0.06 to 0.17 g COD-CH_4 g^{-1}VSS d^{-1} reported elsewhere for self-inoculation (Barbosa & Sant'Anna, 1989; Draaijer *et al.*, 1992). The increase of SMA in RW is attributed to the enrichment of biomass in metal ions as indicated

by the nutrient content. The beneficial effect of metal ions on the activity of methanogens at a moderate concentration has unambiguously been established (Espinosa *et al.*, 1995; Hoban & Van der Berg, 1979; Maqueda *et al.*, 1998). The higher concentration of metal ions in the biomass of RW probably comes from the WEMOS as such, and through increased sedimentation of metal ions already present in the wastewater by the WEMOS. Indeed, chemical coagulation prior to sedimentation improves the retention of metals compared to a natural sedimentation (Cisneros & Meija, 1997).

The presence of active acidogens in RW was indicated by the more rapid decrease of pH and increase of VFA in the effluent of this reactor from day 36. This is consistent with the higher SAA of the biomass of RW. Vanderhaegen *et al.* (1992) and Grootaerd *et al.* (1997) reported that an energy rich substrate like carbohydrate favours the rapid growth of acidogens. Unfortunately such carbohydrates are generally scarce in domestic wastewater arriving at the treatment plants since they are metabolised in the sewer system (Verstraete & Vandevivere, 1999). It therefore appears that the carbohydrates available in the WEMOS (Table 1) contributed to the increase of the SAA of the biomass from RW.

Although granular growth was not visually observed during the 22 weeks of operation, aggregated and filamentous cells dominated the biomass of RW as shown by SEM (Figure 8). The origin of the aggregated bacteria was examined in detail by measuring the EPS content of the biomass grown in both reactors. Moreover the possible presence of specific microorganisms like *Methanosarcina* was checked since they naturally grow in aggregated form. The investigations indicated that biomass from RW and RC presented a similar amount of EPS. It is therefore quite unlikely that EPS was the decisive factor in promoting the aggregated biomass observed in RW. The microbial examination of the samples revealed no *Methanosarcina*-like bacteria, although they had ample time to grow on a suitable substrate. Yet a considerable amount of *Methanosaeta* were

culturable. The WEMOS itself could have therefore contributed to the aggregation of bacteria. Indeed, adsorption of WEMOS on the surface of the dispersed bacteria and neutralisation of their surface charge (N'Dabigengesere *et al.*, 1995) can occur by the patch charge mechanism (Gassenschmidt *et al.*, 1995). The capability of either synthetic or natural polymers to promote such an aggregation of anaerobic cells to form granules has been reported before (El-Mamouni *et al.*, 1998; Imai *et al.*, 1997; Wirtz & Dague, 1996).

Both the microbial enumeration and SEM examination suggest that the growth of the filamentous cells was enhanced in the RW reactor. This is of great interest since filamentous cells play an important role in granule formation (Dubourguier *et al.*, 1988; Hulshoff Pol *et al.*, 1988; Wiegant & De Man, 1986) specially by forming the internal core of the granule (Mac Leod *et al.*, 1990; El-Mamouni *et al.*, 1997). Hence it appears that the continuous supply of WEMOS in a UASB reactor treating domestic wastewater can contribute positively to the start-up of granules formation.

CONCLUSIONS

The goal of this work was to enhance the start-up of a self-inoculated UASB reactor treating raw domestic wastewater. The addition of 2 ml WEMOS (2.5%) l^{-1} of wastewater (without primary sedimentation) before passing through the reactor allowed to shorten the biological start-up of the reactor by 20%. The addition of WEMOS also allowed the aggregation of coccoid bacteria and growth of microbial nuclei, precursors of anaerobic granulation. WEMOS does not affect biogas production intensely, so enough that it may not be economic to continue to dose it after biogas production has started. The use of WEMOS to improve the start-up of self-inoculated reactors can be quite promising in tropical regions where the trees of *M. oleifera* grow easily. In these countries, there is currently a real need to apply an inexpensive anaerobic treatment system such as the UASB reactor to treat domestic wastewater.

ACKNOWLEDGEMENTS

This research was partially supported by a grant from the Government of Côte d'Ivoire. The authors are grateful to Mr Doulaye Kone for providing the seeds of *M. oleifera*. We also thanks Ms Rita Van Driessche for her kind help during SEM analysis.

CHAPTER IV

START-UP OF A SELF-INOCULATED UASB REACTOR TREATING DOMESTIC WASTEWATER - PART III. MICROBIOLOGICAL ASPECTS

Kalogo Y. & Verstraete W.

A modified version of this text was published as:

Kalogo Y., Rosillon F., Hammes F. & Verstraete W. (2000) Effect of a water extract of *Moringa oleifera* seeds on the hydrolytic microbial species diversity of a UASB reactor treating domestic wastewater. *Letters in Applied Microbiology* **31**, 259-264

START-UP OF A SELF-INOCULATED UASB REACTOR TREATING DOMESTIC WASTEWATER - PART III. MICROBIOLOGICAL ASPECTS

Abstract - The effect of a continuous supply of a water extract of *Moringa oleifera* seeds (WEMOS) on the hydrolytic microbial population of biomass grown in mesophilic upflow anaerobic sludge blanket (UASB) reactors treating domestic wastewater was investigated. The WEMOS-treated sludge had seemingly a wider diversity, with *Enterobacter* and *Klebsiella* as dominant hydrolytic bacteria, compared to the control sludge. Additional tests indicated that various hydrolytic bacteria could degrade WEMOS. It appeared that a continuous supply of WEMOS to an anaerobic digester, treating domestic wastewater, increased the diversity of hydrolytic bacteria and therefore enhanced the biological start-up of the reactor.

Key words: Anaerobic sludge, diversity, Domestic wastewater, Hydrolytic, UASB, WEMOS

INTRODUCTION

Moringa oleifera is a tropical plant belonging to the family Moringaceae. The seeds from this plant contain active coagulating agents characterised as dimeric cationic proteins having a molecular weight of 13 kDa and an isoelectric point between 10 and 11 (N'Dabigengesere *et al.*, 1995). Over the last five years there has been a growing interest for using the water extract of *M. oleifera* seeds (WEMOS) as a natural coagulant to treat drinking water (Folkard & Sutherland, 1994; Muyibi & Evison, 1995; N'Dabigengesere *et al.*, 1995; N'Dabigengesere & Narasiah, 1996).

The reliability of WEMOS in this field has motivated investigations into primary treatment of industrial and domestic wastewater (N'Dabigengesere & Narasiah, 1998b). Although these investigations showed good removal of suspended solids it was noticed that an extra chemical oxygen demand (COD) was released in the supernatant fluid. It appears therefore that, in the near future, WEMOS could be a potential challenger of synthetic coagulants (e.g.

iron chloride, aluminium etc) for primary treatment of wastewaters, provided the secondary treatment is biological.

While natural coagulants are biodegradable and *M. oleifera* is known to be not toxic to humans or animals (Berger *et al.*, 1984; Grabow *et al.*, 1985) its effect on microorganisms involved in anaerobic treatment processes in general and upflow anaerobic sludge blanket (UASB) reactors in particular has not yet been reported. It is, however, important to understand the possible implications of WEMOS on a biological treatment system since the substrate composition influences the digester communities (Britz *et al.*, 1994; Howgrave-Graham *et al.*, 1994). The latter then determine the digester performance (Wu *et al.*, 1993). The present study was conducted to examine the effect of a continuous supply of WEMOS on the microbial diversity of a UASB reactor and the importance of the diversity on the performance of the reactor. The study was focussed on the hydrolytic bacteria in view of the important role played by these organisms in the anaerobic digestion process. Hydrolytic bacteria carry out the first phase of the anaerobic digestion process by converting proteins, fats and polysaccharides respectively into amino-acids, glycerol and long chain fatty acids, and monosaccharides. Low hydrolysis can have a severe impact on the overall anaerobic process leading to inefficient methanogenesis (Noike *et al.*, 1985).

MATERIALS AND METHODS

Anaerobic sludge samples

Two anaerobic sludge samples labelled anaerobic control (AC) sludge and anaerobic WEMOS (AW) sludge were examined in this study. Each sample of flocculent sludge originated, respectively, from two laboratory-scale UASB reactors operating in parallel. The reactors, with a volume of 2.3 litre each, were self-inoculated. They operated for 22 weeks at 29 °C, with a hydraulic retention time (HRT) of 4 hours. During the entire experimental period the reactor containing AC sludge treated domestic wastewater. The reactor containing AW sludge treated domestic wastewater supplemented with

WEMOS. In this case, 2 ml of WEMOS (2.5 g powder per 100 ml water) were added daily per litre of wastewater fed to the reactor. This dose increased daily the COD of the raw wastewater by 50 mg l^{-1}.

Enumeration and isolation of hydrolytic bacteria

Hydrolytic bacteria were cultivated on Brain Heart Infusion (BHI) agar medium (Oxoid, Basingstoke, UK). The sludge samples, taken from the reactors at the end of the 22nd week of operation, were serially diluted in triplicate and plated. The plates were incubated at 37 °C for 48 hours and enumeration was done from the highest dilution yielding a count more than 30 but less than 300 colonies. Subsequently, the colonies were selected and re-plated on new BHI agar plates in order to obtain pure cultures. Prior to identification, a gram staining and a spore-forming test were performed (Jenkins *et al.*, 1993).

Identification of hydrolytic bacteria

Based on preliminary observations (gram staining and spore forming test) the strains were characterised by using different API kits (Bio Mérieux SA, Marcy-l'Etoile, France). The results were further verified by plating the identified strains on specific growth media described by Atlas (1993).

Calculations

The hydrolytic bacterial community of the sludge samples was analysed by calculating Shannon's diversity index, H' and the species equitability J' (Atlas *et al.*, 1991). H' and J' were calculated according to the following relations:

$$H' = \left(\frac{C}{N}\right) \times \left[N \times Log_{10}(N) - \sum_{i=1}^{n} n_i \times Log_{10}(n_i) \right]$$

$$J' = \frac{H'}{H_{max}}$$

Where:

C = 3.3219

N = total number of isolates

n_i = total number of isolates in the i^{th} taxonomic group

H_{max} = maximal value of H' for a given sample.

Growth of hydrolytic bacteria on moringa agar

In this additional test, the possibility for hydrolytic bacteria to use WEMOS as a growth factor was examined. The lyophilisate of WEMOS was used as a medium (moringa agar) to grow hydrolytic bacteria. Moringa agar was composed of 0.5% (w/v) lyophilisate of WEMOS and 4% (w/v) noble agar (Difco, Detroit, USA). The control medium was made of 4% (w/v) noble agar. The pH of the both media was adjusted to 7 and they were autoclaved for 15 minutes. Pure cultures of 17 bacterial strains were tested. The strains were provided by the Fondation Universitaire Luxembourgeoise, Arlon, Belgium.

Hydrolytic activity in relation to the microbial diversity

The objective of this test was to examine the relationship between the microbial diversity and the hydrolytic activity of a consortium of bacteria. The test was carried out in batch reactors labelled C, BW and BC with a working volume of 0.5 litre each. The reactors BW and BC were inoculated with 200 ml mixed liquor (concentration 4 10^7 cfu ml^{-1}) prepared with the strains isolated from AW sludge and AC sludge respectively. The reactor C was not inoculated and served as control. Three reactors were fed with 300 ml (0.7 g COD l^{-1}, pH 7.2) of synthetic wastewater. The wastewater consisted of, in terms of COD, 58% meat extract (Oxoid, Basingstoke, UK), 5% starch (Cabbac, Bruxelles, Belgium), 12% vegetable oil (GB N.V., Bruxelles, Belgium), 6% sucrose (Aldrich, Milwaukee, USA) and 19% cellulose (Merck, Darmstadt, Germany). The hydrolytic activity of the consortium of bacteria was assessed by measuring the change in pH evolution according to time.

When the pH decreased, it was brought back to the initial level by titrating with a 0.1 N NaOH solution (Vel, Leuven, Belgium) (Vanderhaegen *et al.*, 1992).

Analytical techniques

Carbon, nitrogen, phosphorus and metal ions were determined in accordance with standard methods (APHA, 1992).
Glucose was measured by the glucose oxidase reaction using a biochemical analyser (Model 2500 Select, Biorad).
Free amino acids were determined with the Biotronik LC 3000 (Merck, Belgolabo) instrument according to the procedure described by Ooghe (1983).

RESULTS AND DISCUSSION

Isolation and identification of bacteria from AC sludge and AW sludge sampled on week 22 resulted in the identification of 7 species of hydrolytic bacteria presented in Table 1 and Figure 1. Prior to identification, preliminary examinations indicated that the species from AW sludge were divided between gram positive (43%) and gram negative (57%). However, all the species from AC sludge were gram negative. Only one of the overall species isolated was a spore-forming bacterium, which originated from AW sludge.

There was a coccoid (Figure 1), gram positive, non-spore-forming bacterium (morphologically resembling Micrococcus) which could not be indentified by the API test.

AC and AW sludge presented a total bacterial number of 3 10^7 cfu ml^{-1} and 4 10^7 cfu ml^{-1} respectively. Although the plate count technique is known to be unsuitable for exact quantification of bacterial populations (Amann *et al.*, 1998), this method appeared to be adapted to the context of this study. Indeed the plating method allows the direct recovery of strains for the identification step. The concentration of microorganisms in both sludge samples was comparable to the concentration of $10^6 - 10^9$ cfu ml^{-1} commonly found for

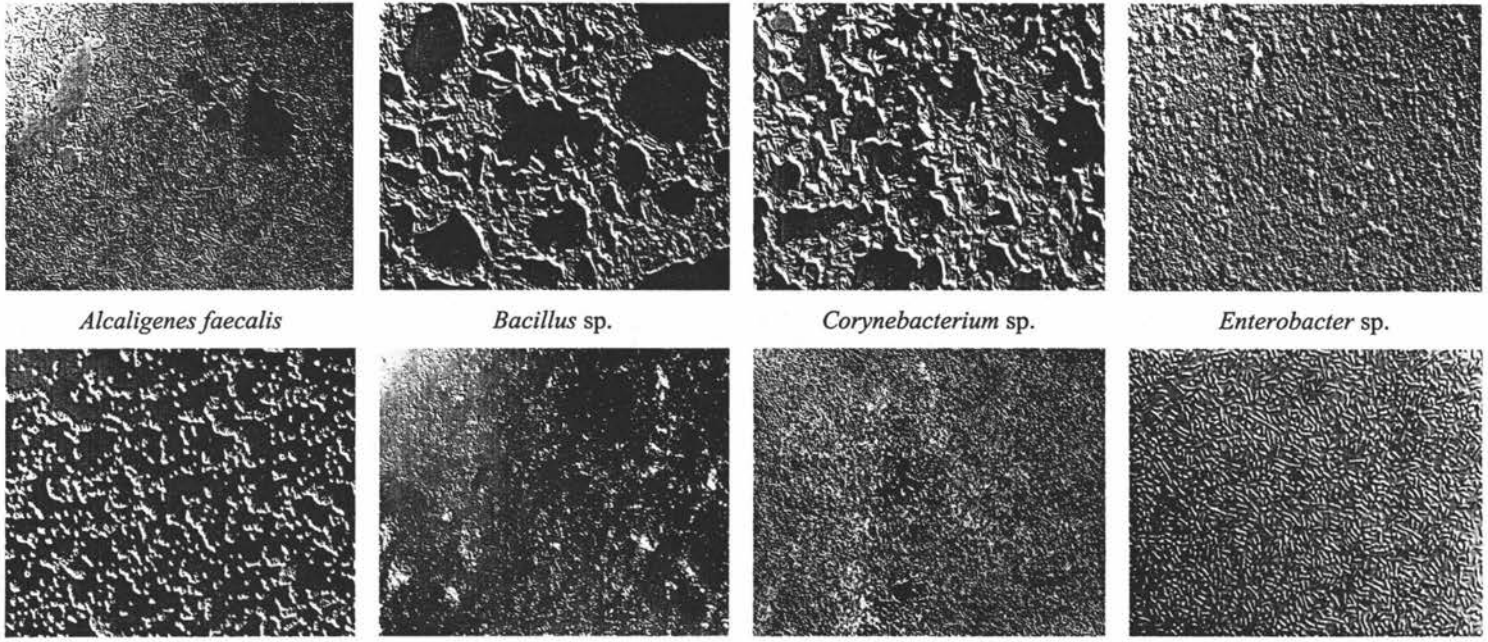
hydrolytic bacteria from mesophilic anaerobic domestic sewage sludge when plated (Crowther & Harkness, 1975; Zeikus, 1979). The microbial diversity of both sludge samples could be estimated by the shannon index (Pielou, 1966).

Table 1. Characteristics, diversity index and equitability of microorganisms isolated from anaerobic control (AC) and WEMOS (AW) sludge

Gram	Spore	Family	Species	AC sludge	AW sludge
-	NSFB	Uncertain	*Alcaligenes faecalis*	(3)	n.d.
+	SFB	Bacillaceae	*Bacillus* sp	n.d.	(6)
+	NSFB	Coryneform	*Corynebacterium* sp	n.d.	(4)
-	NSFB	Enterobacteriaceae	*Enterobacter* sp	(25)	(12)
+	NSFB	n.i.	n.i.	n.d.	(6)
-	NSFB	Neisseriaceae	*Moraxella* sp	n.d.	(1)
-	NSFB	Pseudomonaceae	*Pseudomonas* sp	(5)	(2)
-	NSFB	Enterobacteriaceae	*Klebsiella pneumoniae*	n.d.	(9)
Dilution factor				5	5
Total isolates				33	40
Total species				3	7
Diversity index (H')				1.030	2.5077
Equitability (J')				0.2042	0.4712

Note: SFB = Spore forming bacterium; NSFB = Non-spore forming bacterium; n.d. = Not detected; n.i. = Not identified; ( ) = Data between bracket correspond to the number of isolates.

Figure 1. Morphostructure of micro-organisms isolated from anaerobic control (AC) and WEMOS (AW) sludge



Alcaligenes faecalis | *Bacillus* sp. | *Corynebacterium* sp. | *Enterobacter* sp.

Not identified | *Moraxella* sp. | *Pseudomonas* sp. | *Klebsiella pneumoniae*

The proportions of the different species in the samples showed that *Enterobacter* were the dominant hydrolytic bacteria (75%) of AC sludge. Although this genus was also dominant in AW sludge, it had a lower proportion (30%) compared to that observed in AC sludge. This was assumed to be due to competition between *Enterobacter* and the other species growing in AW sludge, especially *Klebsiella,* since both presented the highest proportion. AW sludge showed a wider microbial diversity compared to AC sludge (Table 1) since an H' value near 0 represents a community with low diversity and a value nearer 4 represents a community with high diversity (Atlas *et al.*, 1991). Similarly, the equitability value was better for AW sludge than AC sludge. Indeed a J' value near 1 indicates an even distribution and a value close to 0 corresponds to an uneven distribution of the isolates within the taxa of the community. Both reactors from which the two samples were taken operated under the same conditions (temperature and HRT), which were moreover kept constant. In view of this fact, the low diversity observed in AC sludge cannot be attributed to the effect of stresses such as temperature or HRT fluctuations (Zeikus, 1979). It is likely due to the poor composition of a substrate like domestic wastewater (Verstraete & Vandevivere, 1999). Such a substrate with a low variety of carbon sources can give rise to a microbial population characterised by a low diversity while a substrate with various carbon sources favours high microbial diversity (Britz *et al.*, 1994). Hence it appears that the presence of carbohydrates and other carbon sources in WEMOS (N'Dabigengesere *et al.*, 1995; Okuda *et al.*, 1999) substantially contributed to the high diversity of AW sludge.

The capability of bacteria to grow on moringa agar was underlined by an additional test. The results of this test (Table 2) indicated that many bacteria commonly present in domestic wastewater are able to consume WEMOS. Among those bacteria, *Enterobacter* and *Klebsiella* were the easiest to grow. This further demonstrates that competition between these two species could be the reason for the reduced proportion of *Enterobacter* in AW sludge as assumed above. Gram positive bacteria could grow on moringa agar, but very slowly. Except for *Pseudomonas aeruginosa*, no bacteria grew on the control media. This indicates that moringa was the main growth factor for the strains.

Table 2. Strains grown on moringa agar at 37 °C

Strains	Aspects of the colonies after 24 h	Aspects of the colonies after 48 h	Aspects of the colonies after 7 d
Gram -			
Citrobacter freundii	(+) wet small colonies	(+) wet small colonies	
E.coli 1	(+) dry small colonies	(+) dry small colonies	
E.coli 2	(+) dry small colonies	(+) dry small colonies	
E.coli 3	(+) dry small colonies	(+) dry small colonies	
E.coli ATCC 25922	(+) dry small colonies	(+) dry small colonies	
Enterobacter cloacae	(+) mucous colonies	(++) mucous colonies	
Enterobacter aerogenes	(+) mucous colonies	(++) mucous colonies	
Klebsiella oxytoca	(+) mucous colonies	(++) mucous colonies	
Klebsiella pneumoniae	(+) mucous colonies	(++) mucous colonies	
Pseudomonas aeruginosa	(+) dry colonies, spread and green	(+) dry colonies, spread and green	
Salmonella 1	(+) very fine colonies, starry, dry	(+) very fine colonies, starry, dry	
Salmonella 2	(+) very fine colonies, starry, dry	(+) very fine colonies, starry, dry	
Salmonella 3	(+) very fine colonies, starry, dry	(+) very fine colonies, starry, dry	
Gram +			
Staphylococcus aureus 2	(-)	(-)	(+) very small colonies
Staphylococcus aureus 3	(-)	(-)	(+) very small colonies
Staphylococcus aureus 4	(-)	(-)	(+) very small colonies
Staphylococcus aureus 6	(-)	(-)	(+) very small colonies

Note: (+) = Positive growth; (++) = Enhanced growth; (-) = No growth.

Table 3. Composition of the lyophilisate of water extract of *Moringa oleifera* seeds (WEMOS), 2.5% powder in 100 ml water

Parameter	Value (mg g^{-1})
C	419.0
N	95.00
P	1.940
Ca	0.760
Mg	1.940
K	11.60
Na	0.430
Ag	0.038
Fe	0.260
Mo	0.362
Ni	0.024
Zn	0.066
Cr	0.020
Glucose	46.50
Free amino acids	Amino acids (g per 100 g proteins)
Aspartic acid	0.114
Serine	0.040
Asparagine	0.125
Glutamic acid	0.453
Glutamine	0.025
Glycine	0.011
Alanine	0.044
Valine	0.016
Methionine	0.009
Isoleucine	0.007
Leucine	0.018
Histidine	0.007
Lysine	0.025
Arginine	0.370
Total free amino acids	1.260

Note: Threonine, Proline, Cystine, Tyrosine, Phenylalanine, Tryptophan and Ornithine were detected as trace.

The direct plating of domestic wastewater on moringa agar at 37 °C led to an average total count of 1.5 10^4 cfu ml^{-1} after an incubation time of 72 hours. The above observations are explained by the composition of moringa (Tables 3) which contains some easily degradable carbon sources such as glucose and free amino acids (mainly glutamic acid, arginine, asparagine and aspartic acid). It is therefore postulated that the continuous supply of WEMOS contributed to improve the hydrolytic microbial diversity of the AW sludge.

In order to understand the impact of the diversity on the performance of a reactor, a second test was carried out. The result (Figure 2) shows a rapid and more pronounced decrease of pH in the BW reactor compared to the BC

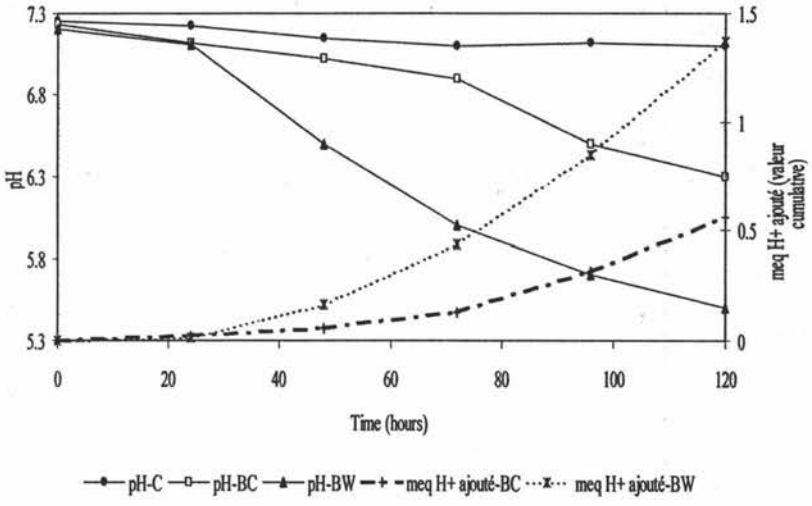


Figure 2. Hydrolytic activity in relation to the hydrolytic microbial diversity

C = control reactor (without inoculum) BC = reactor inoculated with mixture of *Alcaligenes feacalis*, *Enterobacter* sp and *Pseudomonas* sp (concentration of the mixture = 4 10^7 cfu ml^{-1}) BW = reactor inoculated with mixture of *Bacillus* sp, *Corynebacterium* sp, *Enterobacter* sp, *Moraxella* sp, *Pseudomonas* sp, *Klebsiella pneumoniae* and the species not identified (concentration of the mixture = 4 10^7 cfu ml^{-1}) The meq H^+ express the amount of NaOH (0.1 N) added to bring back the pH to its initial value.

CONCLUSIONS

The hydrolytic bacterial community of two sludge samples from two UASB reactors operated in parallel and treating respectively domestic wastewater and domestic wastewater mixed with WEMOS was investigated. In the presence of WEMOS the sludge had a wider diversity. It is concluded that the continuous supply of WEMOS in domestic wastewater has the advantage of improving the hydrolytic microbial diversity of a UASB reactor. The diversity can contribute as such to enhancing the biological start-up of the reactor.

ACKNOWLEDGEMENTS

This research was partially financed by the government of Côte d'Ivoire through a scholarship. The authors gratefully acknowledge Dr. Paul Vander Borgth (Fondation Universitaire Luxembourgeoise, Arlon-Belgium) for providing the bacteria identification material. The authors thank Mr Henri G. Sonan Kouadio for his technical assistance.

CHAPTER V

GRANULATION IN UPFLOW ANAEROBIC REACTOR TREATING DOMESTIC SEWAGE - EFFECT OF HYDRODYNAMICS AND FEED COMPOSITION

Kalogo Y. & Verstraete W.

A modified version of this text is submitted for publication as:

Kalogo Y. & Verstraete W. Granulation in upflow anaerobic reactor treating domestic sewage - effect of hydrodynamics and feed composition. *Water Research*

GRANULATION IN UPFLOW ANAEROBIC REACTOR TREATING DOMESTIC WASTEWATER - EFFECT OF HYDRODYNAMICS AND FEED COMPOSITION

Abstract - Granular sludge formation on domestic wastewater was studied in upflow anaerobic reactors. The effects of reactor hydrodynamics and addition of an energy rich substrate, e.g. molasses, were investigated. Hydrodynamic tests showed that an increase of upflow velocity (V_{up}) lead to an expansion of the sludge bed. The relative expansion (RE) of the sludge bed evolved from 20% (V_{up} = 1 m h^{-1}) to 85% (V_{up} = 5 m h^{-1}) when the reactor was inoculated with 3.5 g suspended solids (SS) l^{-1} reactor. Yet the RE of the sludge bed was dependent on the V_{up} applied and the amount of biomass used to inoculate the reactor. The flow pattern in the reactor, as determined by NaCl tracer experiments, indicated a moderate mixing (d = 0.088 < 0.1) at V_{up} of 1 m h^{-1} and a high mixing (d = 0.309 > 0.1) at V_{up} of 5 m h^{-1}. The results of continuous operation of the reactor showed that at V_{up} of 1 m h^{-1} no granulation was observed although 21% of the chemical oxygen demand (COD) of the feed consisted of COD-molasses. Granulation occurred when the V_{up} was increased to 5 m h^{-1}. The results suggested that good mixing in the reactor was the key factor for granular sludge formation. Granules grown in the non-supplemented control as well as in the molasses fed reactor had similar size (1 mm on average). Scanning electron microscopy (SEM) examination showed that the inner part of the granules consisted mainly of rod shaped bacteria resembling *Methanosaeta*. The molasses fed reactor had a much higher biogas production and biomass specific activity.

Keywords: Anaerobic digestion, domestic wastewater, granulation, upflow velocity.

INTRODUCTION

The upflow anaerobic sludge blanket (UASB) reactor has been applied in many regions to treat domestic wastewater during the last two decades because

it offers several advantages (e.g. low design and operation costs, low space requirement and little excess sludge production). Based on these advantages and the encouraging results observed, the UASB reactor appears today as a robust technology for domestic wastewater treatment (Seghezzo *et al.*, 1998). However, UASB technology is frequently confronted with poor granular sludge formation when it is used to treat domestic wastewater (Kalogo & Verstraete, 1999). So far, granular sludge formation on domestic wastewater has apparently been limited to the report of Barbosa & Sant'Anna (1989). In most of the cases, the biomass grown on domestic wastewater remained in the form of flocculent sludge. One may have an effectively working reactor with a flocculent sludge bed, yet the biomass is easily washed out when a high hydraulic load is applied and this can cause system failure. In order to make the UASB technology more reliable it is advantageous to promote the aggregation of bacterial cells in the form of granules (Lettinga *et al.*, 1983; Schmidt & Ahring, 1996).

Energy rich carbohydrates have been reported to be essential for the rapid growth of granular sludge in UASB reactors (Schmidt & Ahring, 1994; Thaveesri *et al.*, 1995a; Grootaerd *et al.*, 1997). About 20 to 27% of the chemical oxygen demand (COD) of the influent should be in the form of energy rich carbohydrates (Thaveesri *et al.*, 1995b; Grootaerd *et al.*, 1997). In domestic wastewater, carbohydrates are in the range of 10-40 mg on a total of 210-740 mg COD l^{-1} (Henze *et al.*, 2000). They represent therefore about 5% of the COD. The low content of domestic wastewater in carbohydrates could therefore explain the difficulty of granulation on such a wastewater (Verstraete & Vandevivere, 1999).

In other respects, Noyola & Morena (1994) have reported that flocculent anaerobic sludge can be converted to a relatively active granular sludge in a batch reactor with only hydraulic stress. O'Flaherty *et al.* (1997) observed that granulation in a volatile fatty acid (VFA)/ethanol-fed reactor was due to an increase of upflow velocity (V_{up}) in the reactor. Conventionally the UASB reactor operates at a low V_{up} ($\leq$ 1 m h^{-1}). Low V_{up} is due to low hydraulic

loading rate (HLR), which has been reported not to promote granule formation (Lettinga, 1995). With concentrated agroindustrial wastewater, the low HLR is compensated by the high biogas production, e.g. 5-6 m^3 CH_4 per m^3 reactor per day (Pette & Versprielle, 1981; Grusenmeyer & Pipyn, 1987; Souza *et al.*, 1992). The latter then contributes positively to the increase of the turbulence in the reactor. In contrast, the biogas production during anaerobic treatment of domestic wastewater is low, e.g. 0.2-0.6 m^3 CH_4 per m^3 reactor per day, and therefore hardly helps to increase the turbulence in the reactor (Monroy *et al.*, 2000; Kalogo & Verstraete, 1999).

At the beginning of the last decade, a modified version of the UASB reactor, the so-called expanded granular sludge bed (EGSB) reactor, with high V_{up} (5 to 10 m h^{-1}) was introduced by De Man *et al.* (1988) for the direct treatment of domestic wastewater. To date, investigations in this area have been done in reactors inoculated with granular sludge (De Man *et al.* 1988; Vander Last & Lettinga, 1992). The objective of those studies was focused on COD and suspended solids (SS) removal efficiency of the system. No attempts have yet been reported in the line of direct development of granules on domestic wastewater in this type of reactor, although it may offer favourable hydraulic selection conditions.

In this paper, the effect of V_{up} and an energy rich substrate, e.g. molasses, to promote granulation on domestic wastewater, with flocculent anaerobic biomass was examined. Raw molasses is a by-product from the processing of sugar beet.

MATERIALS AND METHODS

Hydrodynamic test

Prior to the operation of the lab-scale reactors, expansion and mixing of the sludge bed were assessed at different V_{up} (from 0.3 to 6 m h^{-1}), in a 2.3 l upflow reactor, with different amounts of inoculum (3.5; 7; 13.5 g SS per litre of reactor). The V_{up} was increased by increasing the recycle flow. The mixing

intensity in the reactor was further examined through a tracer method with NaCl (Vel, Leuven, Belgium) as tracer. The tracer was injected as pulse in the feeding tube, about 10 cm below the entrance of the reactor. The injection volume in the reactor was 23 ml of a solution of 200 g NaCl l^{-1}. This corresponded to an initial concentration (Co) of 2 g NaCl l^{-1} of reactor. In this test, effluent from the reactor was sampled in time. Subsequently, the conductivity was determined. The conductivity was then converted into concentration of NaCl by means of a calibration curve. The conductivity due to the water itself was deducted prior to drawing the residence time distribution (RTD) curve of the tracer. During the two tests, the reactor was fed (influent flow) at the rate of 16 l d^{-1}. This corresponded to a V_{up} of 0.30 m h^{-1}. The reactor was made of glass (Schott-Duran, Germany) with a height of 90 cm and an internal diameter of 5 cm. On top, a decantation sphere of 0.5 l was fitted. The reactor was fed by using peristaltic pumps (Watson Marlow, 313S, Germany).

Calculation

The expansion of the sludge bed was measured when the level of the bed had reached stability. The relative expansion of the sludge bed (RE) was calculated as follows:

$$RE\,(\%) = \frac{(H - L)}{H} \qquad \text{equation 1}$$

Where L = initial level of the sludge bed (no feeding and no recirculation) and H = level of the sludge bed at a given V_{up}.

The fluid mixing patterns were characterised according to the delta impulse on the general dispersion model (Levenspiel, 1972):

$$\left(\frac{\sigma^2}{\tau^2}\right) = 2 \times d - 2 \times d^2 \times \left(1 - e^{\frac{-1}{d}}\right) \qquad \text{equation 2}$$

The dispersion number (d) was calculated by iteration from equation 2.

In case of normal distribution the equation 2 becomes:

$$\left(\frac{\sigma^2}{\tau^2}\right) = 2 \times d \qquad \text{equation 3}$$

The latter allow to directly estimate the dispersion number.

$$\tau = \frac{\sum_{i=1}^{n} (T_i \times C_i)}{\sum_{i=1}^{n} C_i} \qquad \text{equation 4}$$

$$\sigma^2 = \frac{\sum_{i=1}^{n} (T_i^2 \times C_i)}{\sum_{i=1}^{n} C_i} - \tau^2 \qquad \text{equation 5}$$

Where τ = mean of RTD, locates it centre of gravity; σ^2 = variance of the RTD, tells how "fat" the curve is; T_i = sampling time; C = concentration of the tracer at time T_i.

The results were discussed according to Levenspiel (1972) as follows:

d = 0 means no dispersion (ideal plug flow)

$d \leq 0.01$ means low dispersion

$0.01 < d \leq 0.1$ means moderate dispersion

$d > 0.1$ means high dispersion.

The non-dimensional time θ relative to each sampling time T_i was calculated as follows:

$$\theta = \frac{T_i}{T_{th}} \qquad \text{equation 6}$$

The theoretical average residence time (T_{th}) was defined as follows:

$$T_{th} = \frac{V_{reactor}}{(Q_r + Q_i)} \qquad \text{equation 7}$$

Where Q_r = recycle flow rate and Q_i = influent flow rate.

The RTD of the tracer was defined by the function

$$\frac{C}{C_0} = f(\theta) \qquad \text{equation 8}$$

Where C = concentration of the tracer measured in the effluent at time T_i and Co = initial concentration of the tracer injected to the reactor.

In none ideal completely mixed condition in the reactor, the effective volume (V_E) of the reactor can be estimated as follows:

$$V_E = \theta_P \times V_R \qquad \text{equation 9}$$

Where V_R = real volume of the reactor (2.3 l) and θ_P the non-dimensional retention time of the observed peak concentration.

Operation of the reactors

Two laboratory upflow anaerobic reactors labelled control reactor (RC) and molasses addition reactor (RM) were set up. The reactors were operated according to the parameters summarised in Table 1 and fed continuously for 211 days. The reactors had the same characteristics and they were fed with the same pumps as described above. The reactors were fed with a synthetic mixture simulating domestic wastewater (SMSDW) during the whole experiment. The SMSDW was prepared by modifying slightly the composition previously reported by De Souza & Foresti (1996). The SMSDW consisted of, in terms of COD, of 58% meat extract (Oxoid, Basingstoke, UK), 2% starch (Cabbac, Bruxelles, Belgium), 12% vegetable oil (GB n.v., Bruxelles, Belgium), 2% sucrose (Aldrich, Milwaukee, USA), 19% cellulose (Merck, Darmstadt, Germany) and 5% acetate (Vel, Leuven, Belgium). The following minerals were added to the SMSDS: $CaCl_2.2H_2O$ (0.0044 mg l^{-1}), NaCl (0.2490 mg l^{-1}), $CaCO_3$ (0.1200 mg l^{-1}), KH_2PO_4 (0.0400 mg l^{-1}), NH_4Cl (0.0720 mg l^{-1}), $MgCl_2.6H_2O$ (0.0070 mg l^{-1}), $NaHCO_3$ (1.2500 g l^{-1}). All of those chemicals were purchased from Vel.

Table 1. Operating parameters and feed composition

Parameter	Reactor	
	RC	RM
Reactor volume (l)	2.3	2.3
Temperature (°C)	28 ± 2	28 ± 2
Recycle ratio		
Period 1	2	2
Period 2	14	14
Period 3	8	8
Feeding rate (l d^{-1})		
Period 1	16 ± 2	15 ± 2
Period 2	16 ± 2	16 ± 2
Period 3	8 ± 1	8 ± 1
Wastewater pH	7.2 ± 0.2	7.4 ± 0.4
Wastewater alkalinity (mg $CaCO_3$ l^{-1})	725 ± 271	720 ± 261
Wastewater concentration (mg COD l^{-1})	564 ± 81	684 ± 81
Molasses addition (% in terms of wastewater COD concentration)	0	21

Note: Recycle ratio = recycle flow rate (Q_r) over influent flow rate (Q_i)

The reactors were operated in three consecutive periods determined by the V_{up} and the HRT applied. Figure 1 shows an overview of the experimental set-up at low and high V_{up} respectively. The change in HRT was determined by the amount of feed daily supplied to the reactors. During the whole experiment the RM reactor received, in addition, a daily amount of 120 mg COD-molasses per litre of influent. The molasses was obtained from Algist Bruggeman (industry located in the area of Ghent).

The top of each reactor was connected to a gas column. The pH of the liquid in the gas columns was lowered to 4, by addition of HCl (Vel, Leuven, Belgium) in order to avoid the dissolution of CO_2 of the biogas. Methyl orange (Vel, Leuven, Belgium) was added to the liquid as indicator of the biogas level in the columns.

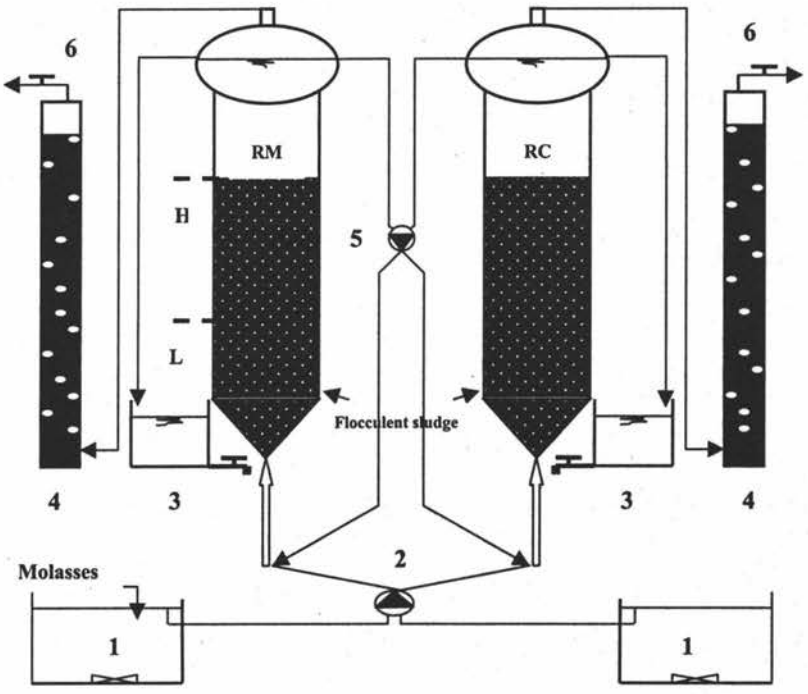


Figure 1. Schematic representation of the experimental set-up operating at high V_{up}, with the sludge bed expanded (not to scale)

1. Influent tanks (raw domestic wastewater) with mixers; **2**. Feeding pump; **3**. Effluent tanks; **4**. Biogas columns full with liquid at pH < 4; **5**. Recycle pump; **6**. Biogas sampling point; **RC**. Control reactor with flocculent biomass; **RM**. Molasses addition reactor with flocculent biomass; **L**. Level (initial) of the sludge bed at low V_{up}; **H**. Level (final) of the sludge bed at high V_{up}.

Inoculum and inoculation

The inoculum used in this study was anaerobically digested sewage sludge obtained from a digester treating primary sewage sludge in Antwerpen (Belgium). The inoculum had a concentration of 38.7 ± 0.7 g SS l^{-1} with 45% in the form of VSS. Based on the results of the hydrodynamic tests, both reactors were inoculated with 200 ml of sludge.

Biomass and methane (CH_4) yields

The yield of biomass ($Y_{biomass}$) was calculated according to Vanderhaegen *et al.* (1992). $Y_{biomass}$ represents the net biomass produced (equals the total biomass harvested minus the initial seed biomass) relative to the total COD (CODt) removed during the experiment (g VSS formed g^{-1} COD removed). The CH_4 conversion coefficient (Y_{CH_4}) was based on the total amount of CH_4 produced per amount of CODt removed.

Metabolic activities, granular sludge characteristics and structure

At the end of the experiment, the specific acidogenic activity (SAA) and the specific methanogenic activity (SMA) of the biomass from each reactor were measured. The SAA was measured according to the technique described by Vanderhaegen *et al.* (1992). The SMA was carried out by the pressure-bottle technique (Shelton & Tiedje, 1984) using acetate as substrate in an anaerobic dilution medium (Valcke & Verstraete, 1983). Both tests were done in batch reactors with grab sample of biomass.

The static settling velocity (V_s) and the size of the granules from the reactors were determined as previously described by Thaveesri *et al.* (1995b). The microbial structure was examined by means of scanning electron microscopy, SEM, (Jeol JSM 840) after fixation of sample sludge with glutaraldehyde solution (Sigma, St. Louis, USA) (Zellner *et al.*, 1991). The sludge samples were examined with a working distance (WD) of 29 mm from the surface of the sample.

Batch experiments

Batch experiments were carried out to examine in detail the effect of the addition of molasses to the reactor. Two batch reactors labelled control batch reactor (BC) and molasses addition batch reactor (BM), each with 2.5 l working volume, were set up. The reactors were operated for 2 months following four successive cycles (C_1, C_2, C_3 and C_4). Each cycle of operation

corresponded to 15 days. During C_1 and C_3, BC was loaded at 8.75 g COD-SMSDW while BM was loaded at 10.6 g COD. 21% of the load of the latter was COD-molasses. During the cycles C_2 and C_4, both reactors were fed with 8.75 g COD-SMSDW only. The reactors were fed with new substrate at the beginning of each new cycle. Total biogas production and SAA of the biomass of the reactors were determined at the end of each cycle.

Analytical techniques

Physico-chemical parameters were determined in accordance with standard methods (APHA, 1992). COD, SS and pH of the effluents were determined 2 to 3 times per week. The pH into the reactor and biogas production was daily monitored. CH_4 and CO_2 in the biogas were analysed with a gas chromatograph (Intersmat IGC 120 MB) connected to a Hewlett-Packard 3390 A integrator. Conductivity was measured with a conductimeter (Consort C833, BRS-Anderlecht, Belgium).

RESULTS

Hydrodynamic test

The effect of V_{up} on the sludge bed is shown in Figure 3. Increase of V_{up} led to a progressive expansion of the sludge bed. $V_{up} \leq 1$ m h^{-1} resulted in no or only a slight expansion ($\leq$ 20%) of the bed and inhomogeneous distribution of water. This created the development of channels through the bed. Intensive mixing of the biomass started from 3 m h^{-1} and improved the expansion of the bed to some 60% when the reactor was inoculated with 3.5 g SS l^{-1}. Mixing was visibly better at higher V_{up} (5 and 6 m h^{-1}). At the latter V_{up} the expansion reached up to 85% in the reactor inoculated with 3.5 g SS l^{-1}. Yet for a given V_{up}, the lower the amount of sludge the better was the expansion (Figure 3). This indicates that good mixing in the reactor depends on the V_{up} applied and on the amount of sludge present in the reactor.

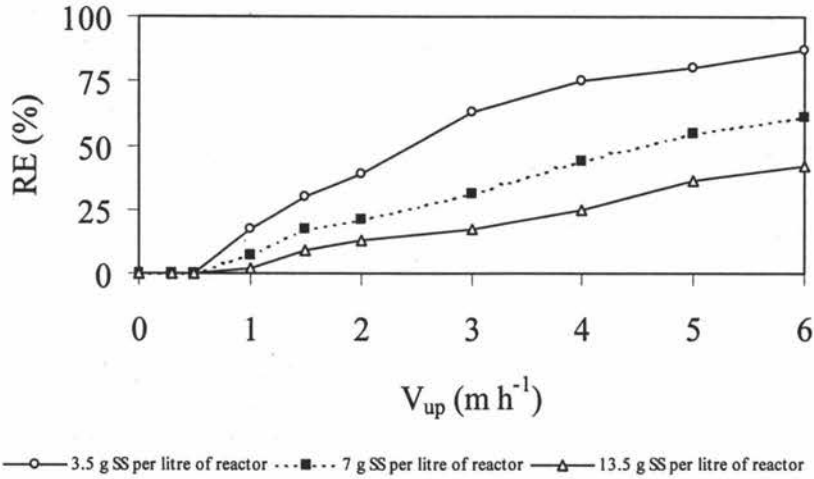
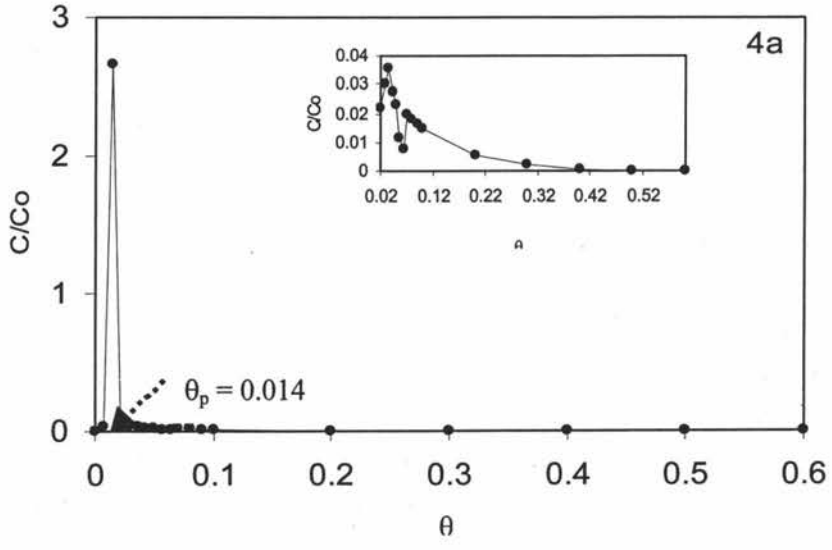


Figure 3. Relative expansion (RE) of the sludge bed versus V_{up} for different amounts of biomass in a 2.3 l upflow reactor

The fluid mixing patterns of the reactors were further investigated at low (1 m h^{-1}) and high (5m h^{-1}) V_{up}. The RTD of the tracer for the two V_{up} applied were different from each other (Figure 4a and 4b). At V_{up} of 1 m h^{-1} the RTD showed an early significant peak ($\theta = 0.014$). The peak observed at $\theta = 0.014$ was significantly higher than 1. The main parameters used to characterise the two patterns are summarised in Table 2.



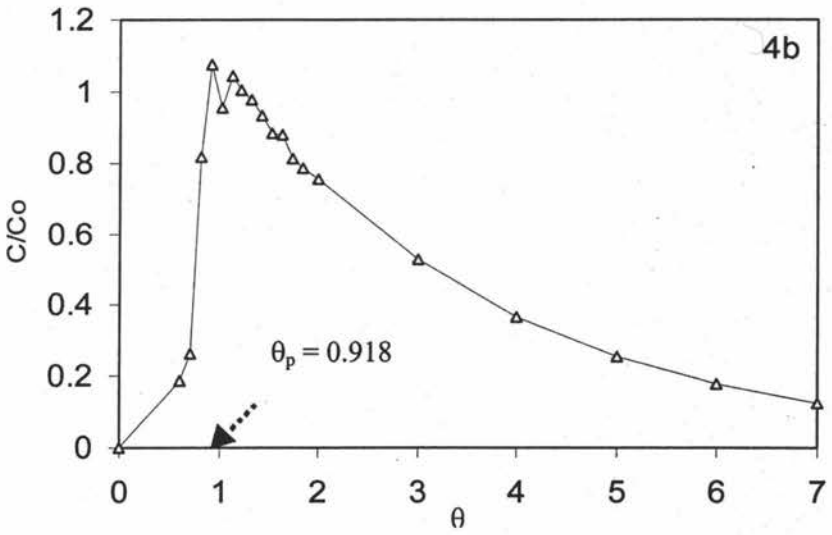


Figure 4. Residence time distribution of the tracer for V_{up} of 1 m h^{-1} (4a) and for V_{up} of 5 m h^{-1} (4b)

Table 2. Main parameters used to characterise the patterns of the tracer

	V_{up}	
Parameter	1 m h^{-1}	5 m h^{-1}
d (equation 2)	0.088	0.309
d (equation 3)	0.082	0.229
T_{th} (min)	70	14
θ_p	0.014	0.918
V_E (l)	0.03	2.11

Period 1 (day 1 to day 60) V_{up} = 1 m h^{-1} and HRT = 3.6 h

Overall performance

During this period the RC reactor was operated with an average volumetric loading rate (B_v) of 3.5 ± 0.4 g COD l^{-1} d^{-1} (Fig. 5a). The supply of the additional 120 mg COD-molasses per litre of the feed of RM reactor resulted in an average B_v of 4.2 ± 0.5 g COD $l^{-1}d^{-1}$ in that reactor (Fig. 5a). The removal

efficiencies of the reactors are plotted on Figure 5b and average efficiencies are given in Table 3. Both reactors showed similar removal efficiencies. The increase of the B_v of the RM reactor with molasses addition did not affect the reactor performance. The biomass in the RM had no difficulty to metabolise the extra COD-molasses supplied. As a consequence, the RM reactor constantly produced more biogas than the RC reactor.

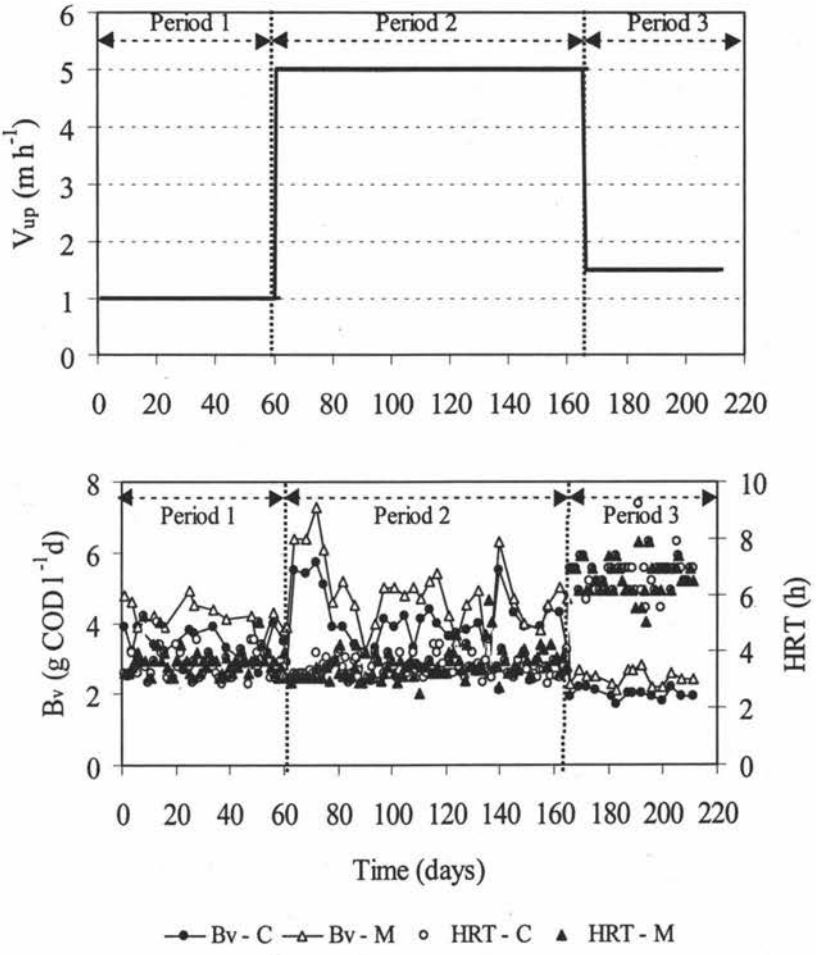


Figure 5a. Upflow velocity (V_{up}), Volumetric loading rate (B_v) and Hydraulic retention time (HRT) as a function of time

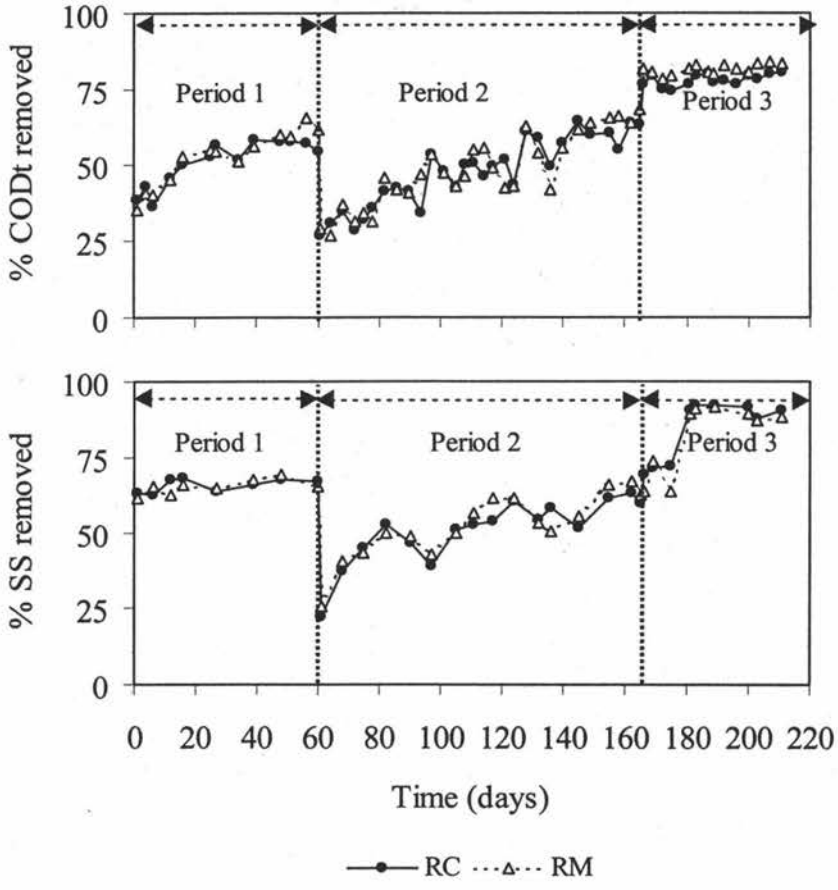


Figure 5b. Removal efficiencies of COD and SS as a function of time

Granular sludge formation

No granulation was observed during period 1. There was no visible mixing of the sludge bed and no distinctive floc formation. As observed during the hydrodynamic tests, the sludge bed in the reactors regularly showed the development of channels. Moreover biogas production sometimes caused the separation of the bed and moving up of the upper part. The latter was then run down in the decanter fitted on top of the reactor.

Table 3. Results of reactor performance and biomass yield

Parameter	Reactor	
	UASB C	UASB M
pH		
In reactor	7.3 ± 0.1	7.2 ± 0.1
Effluent	8.1 ± 0.3	7.9 ± 0.4
Effluent CODt (mg l^{-1})	253 ± 114	294 ± 137
CODt removal efficiency (%)		
Period 1	51 ± 8	52 ± 9
Period 2	47 ± 11	48 ± 12
Period 3	78 ± 2	81 ± 2
Overall	55 ± 16	57 ± 17
SS removal efficiency (%)		
Period 1	66 ± 2	65 ± 2
Period 2	51 ± 11	52 ± 11
Period 3	84 ± 10	82 ± 12
Overall	64 ± 17	64 ± 16
Y biomass (g VSS g^{-1} COD removed)	0.14 ± 0.01	0.19 ± 0.02
VSS/SS ratio in granules		
Period 2	0.27 ± 0.02	0.31 ± 0.01
Period 3	0.21 ± 0.01	0.26 ± 0.03
Settling velocity (m h^{-1})		
Period 2	61.3 ± 22.1	43.2 ± 33.3
Period 3	78.2 ± 31.7	59.7 ± 29.1
Size of granules (mm)		
Period 2	0.73 ± 0.42	0.72 ± 0.31
Period 3	1.21 ± 0.43	1.18 ± 0.58
CH_4 in biogas (%)		
Period 1	75 ± 3	75 ± 4
Period 2	92 ± 1	91 ± 1
Period 3	91 ± 1	90 ± 1
Overall	88 ± 6	87 ± 5
Y methane (l CH_4 g^{-1} COD removed)	0.317	0.305

Period 2 (day 61 to day 165) V_{up} = 5 m h^{-1} and HRT = 3.6 h

Effect on the overall performance

The increase of V_{up} during period 2 (Fig. 5a) negatively affected the SS and COD removal efficiencies due to the expansion of the sludge bed (Fig. 5b and

Table 3). The expansion of the sludge bed sometimes caused wash out of biomass from the reactors. Figure 6 shows the appearance of biomass washed out with effluents of the RM reactor at the beginning of period 2. It indicates a morpho-structure mainly dominated by coccoid bacteria and poor in rod shaped bacteria.

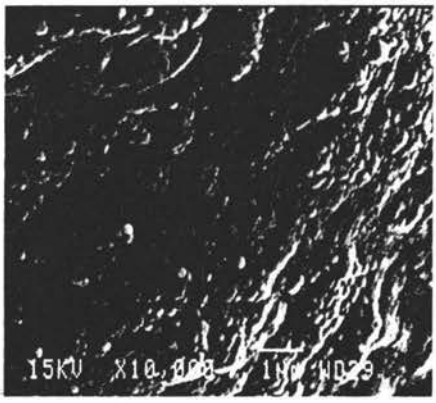


Figure 6. Appearance of biomass washed out at the beginning of period 2 (RM reactor)

Effect on granular sludge formation

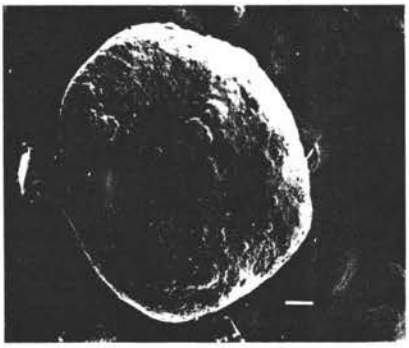
About 24 hours after increasing the V_{up} to 5 m h^{-1} a clear distinctive floc was developed in both reactors. Mixing of the biomass started in the reactors due to the homogeneous distribution of water. This operating condition suppressed the problem of sludge separation and moving up because the biogas could easily leave the liquid phase. Progressively, the sludge bed became denser and fine aggregates, apparently granules, gradually developed in both reactors. The development of granules was confirmed from day 103 after a wash out of biomass (caused by an abrupt re-start up of the reactor after a few hours power cut off) with the effluent of the reactors.

Granular sludge characteristics and structure

At the end of period 2, granule characteristics and structure were examined. Granules from RC showed better settling velocity than those from RM (Table 3). Granules from RC had a lower VSS/SS ratio than those from RM (Table 3).

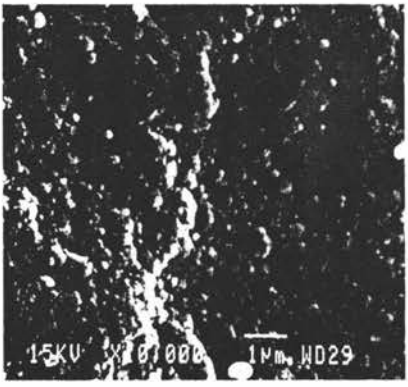
The average size of the granules did not indicate a significant difference between the diameter of both types of granules (Table 3).

Figure 7 shows the SEM of granules harvested from the reactors. The surface layer (C and D) and the inner part of the granules are morphologically different. The surface layer shows coccoid bacteria. The inner part (E and F) indicates mainly rod-shaped bacteria resembling *Methanosaeta* and only a few cocci bacteria.

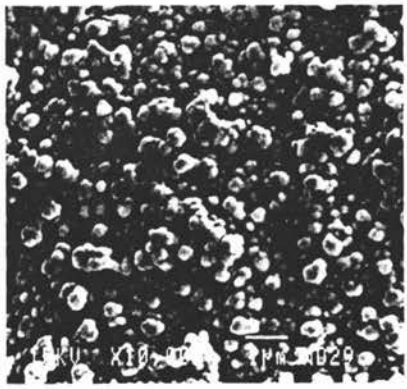


A. Granule from RC (bar = 100 μm)

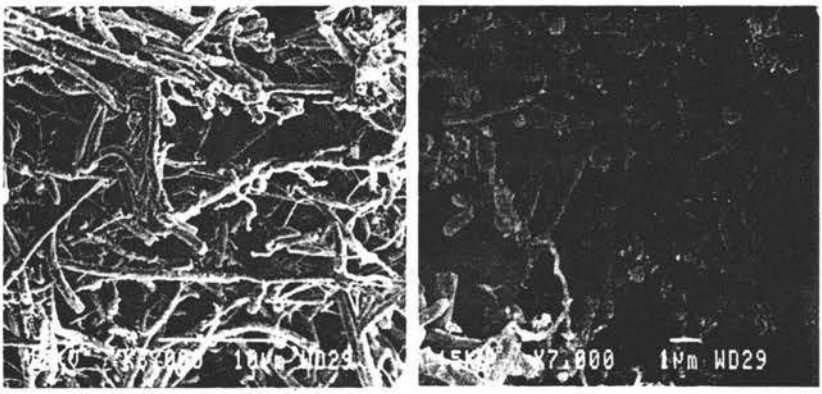
B. Granule from RM (bar = 100 μm)



C. Surface layer of granule from RC



D. Surface layer of granule from RM



E. Inner part of granule from RC **F.** Inner part of granule from RM

Figure 7. Structure of granules harvested from the reactor at the end of period 2

Period 3 (day 166 to day 211) V_{up} = 1.5 m h^{-1} and HRT = 6.7 h

Effect on reactor performance

During period 3, due to the increase of the HRT to 6.7 hours, the daily amount of influent supplied to the reactors was reduced by half compared to the initial amount of period 1 and 2. Consequently, the B_v of the reactors decreased to 2 ± 0.2 g COD l^{-1} d^{-1} for RC and 2.5 ± 0.2 g COD l^{-1} d^{-1} for RM (Fig. 5a).

Figure 5b and Table 3 show that the COD and SS removal efficiency of both reactors were better during period 3 than those of period 1 and period 2. The reactors could regularly reach more than 90% removal efficiency.

Effect on metabolic activities and granular sludge characteristics

Metabolic activities were measured at the end of period 3 (Table 4). The results showed that biomass from RM had higher SAA than that from RC. However, both types of biomass had almost similar SMA. The SAA/SMA ratio indicated that biomass from RM contained specifically more acidogens than that from RC.

Table 4. Specific bacterial metabolic activities of granules harvested at the end of the experiment

Biomass type	SAA (meq $H^+g^{-1}VSS\ h^{-1}$)	SMA (g COD-CH_4 $g^{-1}VSS\ d^{-1}$)	SAA/SMA
RC	0.99 ± 0.03	0.23 ± 0.02	4
RM	2.09 ± 0.05	0.21 ± 0.04	10

Examination of the physical characteristics of the granules revealed an increase of their settling velocity compared to that of period 2. Inversely the VSS/SS ratio of the granules had decreased. Yet granules from RC had the lowest VSS/SS ratio (Table 3). There was no significant difference in granule size (Table 3). Most of the granules had their diameter comprised between 0.6-1.7 mm (Fig. 8). Granules harvested from RM showed slightly higher amounts of large granules compared to those from RC (Fig. 8).

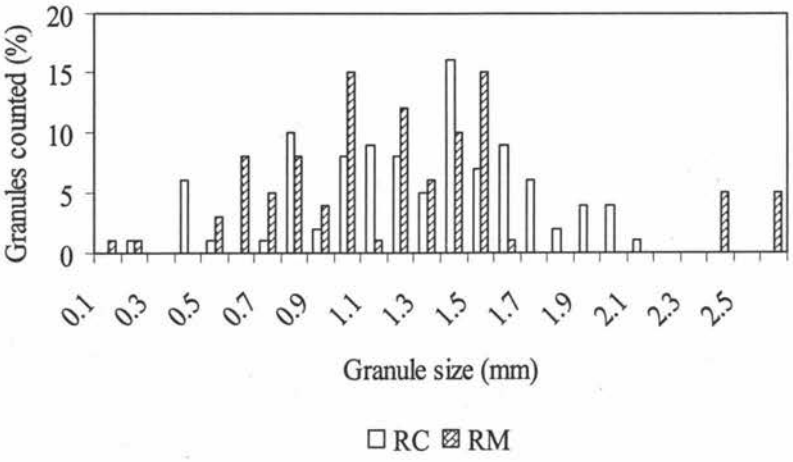


Figure 8. Size distribution of the granules harvested at the end of period 2

Effect of molasses addition (Batch experiment)

The effect of molasses addition was further examined in batch reactors (Fig. 9). The results showed that the BM reactor produced more biogas when molasses

was supplied. Moreover the biomass of the BM reactor showed an increase of SAA when molasses was supplied. There was a drop of both biogas production and SAA of the BM reactor when molasses addition stopped. The BM and BC reactors showed similar biogas production and SAA when no molasses was supplied to the reactors.

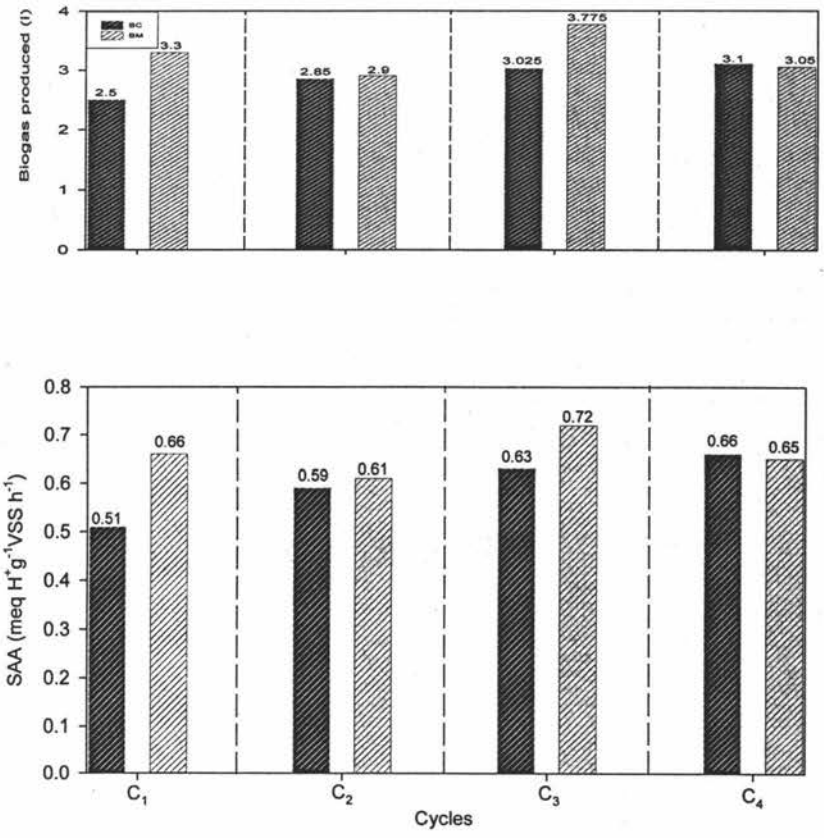


Figure 9. Effect of the addition of molasses on reactor biogas production and biomass activity (batch experiment).

Grey = BM reactor = molasses batch reactor

Black = BC reactor = control batch reactor (fed only with domestic wastewater

C_1 and C_3: cycles with addition of COD-molasses to BM reactor

C_2 and C_4: cycles without addition of COD-molasses to BM reactor

BC)

Duration of $C_1 = C_2 = C_3 = C_4 = 15$ days.

DISCUSSION

The results of the hydrodynamic tests suggest that the mixing intensity in the reactor is depending on the V_{up} (Figure 4a and 4b). The interpretation of the RTD data by the dispersion model should be relativated because the curves as given in Figure 4 obviously do not obey the normal (Gaussian) distribution. Yet the data allow to indicate some overall characteristics. The RTD at V_{up} of 1 m h^{-1}approaches a plug flow trend. The presence of an early significant peak indicates strong short-circuiting from the inlet to the outlet of the reactor. This observation confirms the development of channels through the bed at $V_{up} \leq 1$ m h^{-1}, resulting in inhomogeneous distribution of tracer. Yet the peak was significantly higher than 1. This may be due to the high density of tracer (200 g l^{-1}). Probably the tracer is not properly mixed with the flow of water below the entrance of the reactor. This results in a sudden increase of tracer concentration in the effluent as the reactor displays a plug-flow trend with channels in the bed. The calculation of the effective volume of the reactor at $V_{up} = 1$ m h^{-1} shows that the tracer moves up in a channel of 0.03 ml (Table 2). Boumansour *et al.* (1998) reported that a tracer response curve, after pulse injection, of a plug flow reactor with recirculation flow rate has multiple peaks. The tracer response curve observed in the present work, at V_{up} of 1 m h^{-1}, had only one significant peak. This suggests that the recirculation flow had no strong effect on the dispersion of the tracer. The d value equal to 0.088 at V_{up} of 1 m h^{-1} confirmed that the dispersion in the reactor was moderate.

The RTD at V_{up} of 5 m h^{-1}displays a mixed flow trend. This was confirmed by the calculation of the d value. Indeed, the latter equal to 0.309 was significantly higher than 0.1 and therefore indicates an increase of the mixing intensity in the reactor. The effective volume of the reactor 2.1 l (Table 2) also confirmed that the reactor was well mixed because the total volume was 2.3 l.

During the continuous experiment there was no granular sludge formed when the reactors were operating at the V_{up} of 1 m h^{-1} despite the supply of 120 mg COD-molasses in the RM reactor. About 24 hours after increasing the V_{up} to 5 m h^{-1}, distinctive flocs had established in the reactors. This indicates that the increase of V_{up}, besides the improvement of the dispersion of the substrate, created a shear stress condition in the sludge bed resulting in floc formation. Progressively granules developed in both reactors. This observation demonstrates that adequate hydrodynamic conditions play a major role in promoting granules rather than energy rich substrate, e.g. molasses. The main advantage of the latter is its capacity to improve the activity of the biomass, by increasing the development of acid-formers, as shown by the SAA and the biogas production of RM (continuous experiment) and BM (batch experiment) reactors.

SEM of the biomass washed from the reactors showed a structure poor in rod shaped bacteria. This suggests that the increased velocity tends to exert a selection pressure of aggregate-forming bacteria and wash out of poor settling bacteria. Indeed, among the different microbial nuclei that are potential precursors of anaerobic granulation the rod shaped types had a higher settling velocity (El-Mamouni *et al.*, 1995).

The granules formed in the reactors were different in their physical characteristics. There was no significant difference in granule size. However, granules from RC had a better settling velocity. This is due to the low VSS/SS ratio of granules from the RC reactor, which implies that those granules had a higher density. These observations are consistent with the law of Stokes as follows: $V_o = g\ d^2\ (\Delta\rho)/18\ \mu$, where g = gravity and μ = dynamic viscosity. This relation indeed indicates that the settling velocity (V_o) is proportional to the density (ρ) and the square of the particle diameter (d).

The SEM of the granules shows that their inner part consisted mainly of rod-shaped bacteria resembling *Methanosaeta*. This substantiates previous reports indicating that such bacteria play an important role in granule formation

specifically by forming the internal core of the granule (Mac Leod *et al.*, 1990; El-Mamouni *et al.*, 1997).

During period 3, the removal efficiency of the reactors increased when the HRT and V_{up} increased and decreased respectively. This suggests that a reactor working at high HRT and low V_{up} can profit from a better performance. Van Haandel and Catunda (1997) observed that at temperatures above 20 °C, the higher the HRT, the better was the removal efficiency.

CONCLUSIONS

The study showed that hydrodynamics and feed composition influence the microbial structure of an upflow anaerobic reactor treating domestic sewage. Increasing the upflow velocity of the reactor leads to an expansion of the sludge bed and increases the mixing intensity. It appears that a mixed flow reactor facilitates the development of granules rather than a plug-flow reactor. The supply of an energy rich substrate, e.g. molasses, is not the primary determining factor in promoting granules. Molasses mainly increase the specific activity of the fermentative bacteria. Provided it is available at low cost, the supply of an energy rich carbohydrate as co-substrate during the treatment of domestic sewage in an upflow anaerobic reactor can be recommended in view of the much higher specific activity of the biomass and biogas production in the molasses fed reactor. The higher production of biogas may indeed allow to decrease slightly the recycle flow and may therefore save electrical energy consumption.

ACKNOWLEDGMENTS

This research was partially supported by the government of Côte d'Ivoire through a scholarship. The authors would like to thank Prof. Adrianus Van Haandel for constructive discussions about granular sludge formation. We thank Prof. Jean-Luc Vasel for his suggestions relative to the tracer experiment.

CHAPTER VI

TECHNICAL FEASIBILITY OF THE TREATMENT OF DOMESTIC WASTEWATER BY A CEPS-UASB SYSTEM

Kalogo Y. & Verstraete W.

A modified version of this text was published as:

Kalogo Y. & Verstraete W. (2000) Technical feasibility of the treatment of domestic wastewater by a CEPS-UASB system. *Environmental Technology* **21**, 55-65

TECHNICAL FEASIBILITY OF THE TREATMENT OF DOMESTIC WASTEWATER BY A CEPS-UASB SYSTEM

Abstract - Raw domestic wastewater was treated continuously under laboratory conditions for 170 days by a chemically enhanced primary sedimentation (CEPS) followed by an upflow anaerobic sludge blanket (UASB) reactor. The CEPS was carried out with 70 mg $FeCl_3$ l^{-1} from day 1 to 82 and with 24 ml l^{-1} of the water extract of *Moringa oleifera* seeds (WEMOS) from day 83 to 170. Compared to the natural primary sedimentation (NPS), the CEPS increased the ratio of soluble chemical oxygen demand to volatile suspended solids (CODs/VSS) of the supernatant fluid by a factor of 3 and 10 respectively. Although the $FeCl_3$ increased the CODs/VSS ratio, it caused a decrease of the CODs of the wastewater by a factor 1.4. This resulted in a low influent concentration supplied to the UASB reactor and consequently a low biogas production. However, the reactor achieved 54% removal of total COD (CODt) at a hydraulic retention time (HRT) of 2 hours and a volumetric loading rate (B_v) of 1.4 g COD l^{-1} d^{-1}. The WEMOS, besides increasing the CODs/VSS ratio, allowed also to increase the CODs content of the wastewater by a factor of 2.2. As a consequence, the reactor produced a higher amount of biogas. The reactor achieved 71% removal of CODt at a HRT of 2 hours and a B_v of 4 g COD l^{-1} d^{-1}. The UASB reactor operated without the need to regularly discharge the excess of sludge produced. The implementation of the CEPS can decrease the volume needed by a classical one step UASB reactor by a factor of 0.4. The system had only a modest impact on the removal of total coliform, faecal coliform and faecal streptococci.

Keywords: CEPS, CODs/VSS ratio, $FeCl_3$, *M. oleifera* seeds, raw domestic wastewater, UASB, WEMOS.

INTRODUCTION

In the last ten years, there has been a growing interest for the direct treatment of raw domestic wastewater in upflow anaerobic sludge blanket (UASB) reactors. Several full-scale installations have been built in tropical regions due to their low investment, operation and sludge disposal costs and low space requirement compared to most of the aerobic systems and the other anaerobic systems (Van Haandel & Catunda, 1997). However, despite these advantages, the application of the UASB technology for the direct treatment of raw domestic wastewater still faces major scepticism.

Raw domestic wastewater contains about 45 to 55% of the total chemical oxygen demand (CODt) in the form of suspended solids (SS) (Tchobanoglous & Burton, 1991). During treatment in a UASB reactor, the SS fraction tends to accumulate into the reactor sludge bed since it is hydrolysed very slowly (Rozzi & Verstraete, 1981). The undegraded SS negatively affect the methanogenic activity of the granular sludge (Sayed & Fergala, 1995). In order to overcome the negative effect of the SS, the concept of a two-stage reactor was suggested (Sayed & Fergala, 1995; Wang, 1994). Although this concept is attractive, it needs a third reactor for the stabilisation of the excess sludge regularly discharged from the first reactor. Hence the system appears to be costly and technically complicated. Another approach consists of submitting the wastewater to a natural primary sedimentation (NPS) before feeding the UASB reactor (Elmitwalli *et al.*, 1999; Vieira, 1988). This approach is simple and economically acceptable, but it can take half a day to remove a part of the resistant fraction of the SS (Elmitwalli *et al.*, 1999). In practice, NPS hardly improves the ratio of soluble oxygen demand to volatile suspended solids (CODs/VSS) of the wastewater since it has a minor effect on the colloidal fraction of the SS (Garcia *et al.*, 1998). However, a CODs/VSS ratio of about 10 is one of the key factors to achieve a suitable treatment in a UASB reactor (De Baere & Verstraete, 1982; De Baere *et al.*, 1984). As synthetic and natural coagulants are efficient in SS removal from domestic wastewater within a reasonable period of time (Ndabigengesere & Narasiah,

1998b), it seems interesting to perform a chemically enhanced primary sedimentation (CEPS) rather than a NPS before feeding a UASB reactor. This approach merits a careful investigation since it could lead to the change of the physico-chemical parameters of the wastewater, such as the pH, the alkalinity, the equilibrium COD/N/P etc, which are important factors involved in the anaerobic process (Champiat, 1994).

The general purpose of the present work was to study the technical feasibility of the treatment of domestic wastewater by a CEPS–UASB system. This system should efficiently reduce the negative effect of the SS on the activity of the methanogens. It should also avoid the construction of three reactors, which is mandatory in the two-stage reactor concept, since the primary sludge could be directly treated in a completely stirred tank reactor (CSTR).

The study was conducted with the following specific objectives:

- to investigate the possibility of using synthetic and natural coagulants in pre-treatment of raw domestic wastewater in order to increase the ratio CODs/VSS of the supernatant fluid,
- to assess the performance of a UASB reactor treating a chemically sedimented domestic wastewater with synthetic and natural coagulants,
- to evaluate the hygienic potentiality of the CEPS-UASB system.

The study was focused on the utilisation of $FeCl_3$ and *M. oleifera* seeds as sources of coagulants. The former is a synthetic coagulant widely used for the treatment of wastewaters (Tchobanoglous & Burton, 1991). *M. oleifera* is a tropical plant used for the treatment of some tropical diseases (Kerharo, 1974). The seeds and the husk have been recently shown to be respectively useful as natural coagulant for the treatment of drinking and waste waters (Folkard & Sutherland, 1994; Ndabigengesere & Narasiah, 1998b) and as raw material for the production of high quality activated carbon (Pollard *et al.*, 1995).

MATERIALS AND METHODS

Wastewater

The wastewater used during the whole experiment was raw domestic wastewater collected from the Ossemeersen treatment plant, city of Ghent (Belgium). The main characteristics of the wastewater used for the operation of the CEPS-UASB system are given in Table 1.

The raw wastewater had on average a CODt of 268 mg l^{-1} with a CODs/VSS ratio of 1.4. The COD in the form of suspended solids was 48% of the CODt.

Table 1. Characteristics of the raw domestic wastewater

Parameter (unit)		Average ± standard deviation
pH		7.6 ± 0.2
Alkalinity	(mg $CaCO_3$ l^{-1})	361 ± 44
CODt	(mg l^{-1})	268 ± 44
CODs	(mg l^{-1})	139 ± 34
SS	(mg l^{-1})	128 ± 27
VSS	(mg l^{-1})	100 ± 24
TKN	(mg l^{-1})	37 ± 5
NH_4^+-N	(mg l^{-1})	26 ± 3
Ptotal	(mg l^{-1})	7 ± 3
PO_4^{3-}-P	(mg l^{-1})	4 ± 2
Fe	(mg l^{-1})	1.9 ± 0.1
TC	(Log cfu 100 ml^{-1})	8.0 ± 0.2
FC	(Log cfu 100 ml^{-1})	7.4 ± 0.4
FS	(Log cfu 100 ml^{-1})	6.6 ± 0.2

Coagulants

During the study, $FeCl_3.6H_2O$ (Merck, Darmstadt, Germany) was used as synthetic coagulant. The addition was based on the $FeCl_3$ component. The compound was solubilized in distilled water before use. Since it is totally soluble in water, it was dosed on the basis of mg l^{-1}. The seeds of *M. oleifera* used in the study were obtained from the Centre National des Semences Forestières in Burkina Faso (West Africa). The dry pods of *M. oleifera* were harvested in Fada (Burkina Faso) in March 1993.

Before use, the bark enveloping the seeds was removed and the seeds were ground to powder in a laboratory porcelain mortar (Haldenwanger, Germany). The solution of *M. oleifera* was prepared according to NDabigengesere *et al.* (1995) by adding 5 g of the powder to 100 ml distillate water. The mixture was centrifuged (30 min; 10,000 rpm) and the suspension was filtered (Whatman filter paper n° 42). The filtrate was used during the study and it is called WEMOS (water extract of *M. oleifera* seeds). The dose of the coagulant was expressed in ml l^{-1} instead of mg l^{-1} because only about 25% of the dry matter is dissolved during the extraction of the coagulating active agent of WEMOS (Ndabigengesere *et al.*, 1995).

Experiments

Primary coagulation and sedimentation test

Before starting the operation of the treatment system, a jar test was carried out to determine the optimal dose of each coagulant. The jar test was performed in the Geppert apparatus (type NSR 6, Rührtechnik, Germany). The optimal dose was defined as the dose giving the supernatant fluid with the highest CODs/VSS ratio. The CODs, the pH and the alkalinity of the supernatant fluid were also taken in consideration. The doses of 0; 10; 30; 50; 70; 90 and 120 mg of $FeCl_3$ per litre of wastewater were tested. For the WEMOS, the doses of 0; 0.2; 2; 8; 16; 24; 32 ml per litre of wastewater were tested. One litre sample of the raw wastewater and the needed amount of coagulant were added to one litre beakers. The mixing times were fixed according to previous work (Kalogo, 1995) as follows:

- one minute for rapid mixing at 100 rpm,
- ten minutes for slow mixing at 40 rpm.

After mixing, the liquors were allowed to settle for one hour and the supernatant fluids were taken for analysis. The test was performed 3 times (duplicated sample at each time) with wastewater collected at different days.

The supernatant fluids from pre-treatment with optimal doses of $FeCl_3$ and WEMOS were characterised in order to examine the effect of the coagulants on the rapidly acidifying COD (RACOD), e.g. glucose, and metal ions concentration of the supernatants fluid.

Operation of the CEPS-UASB system

The treatment system consisted of a mixing tank with 20 l working volume, a primary decanter of 20 l and a UASB reactor of 1.2 l. Figure 1 shows the schematic representation of the experimental set-up. The raw wastewater was fed batchwise once a day and mixed with the optimal dose of coagulant. After one hour settling, the supernatant fluid was pumped continuously into the UASB reactor using a peristaltic pump (Watson Marlow, 313S, Germany). In parallel to the CEPS, raw wastewater was submitted to a NPS for 1 hour. The CODs/VSS ratio of the supernatant fluid from the latter treatment was taken as control.

The UASB reactor was made of glass (Schott-Duran, Germany) with a height of 45 cm and an internal diameter of 5 cm. On top, a decantation sphere of 10 cm diameter and a volume of 0.3 l was fitted. The reactor was inoculated with 60 ml of granular sludge obtained from a full-scale UASB reactor of a potato processing industry (Primeur, Belgium). The sludge concentration was 56 g VSS l^{-1}. Since the inoculum was stored during three weeks at 4 °C, reactivation was done before the inoculation of the reactor. This reactivation also allowed the inoculum to adapt to domestic wastewater. The sludge was reactivated during three weeks with a synthetic wastewater simulating domestic wastewater (De Sousa & Foresti, 1996). At the end of the reactivation period the sludge had a specific methanogenic activity (SMA) of

0.21 ± 0.02 gCH_4-COD g^{-1}VSS d^{-1}. The operation of the reactor was divided in two periods. During the first period (day 1 to 82), the coagulant used was the $FeCl_3$. The WEMOS was used in the second period conducted from day 83 to 170. The start up of the reactor in the second period was done immediately after the first period by using the granular sludge harvested after the first period.

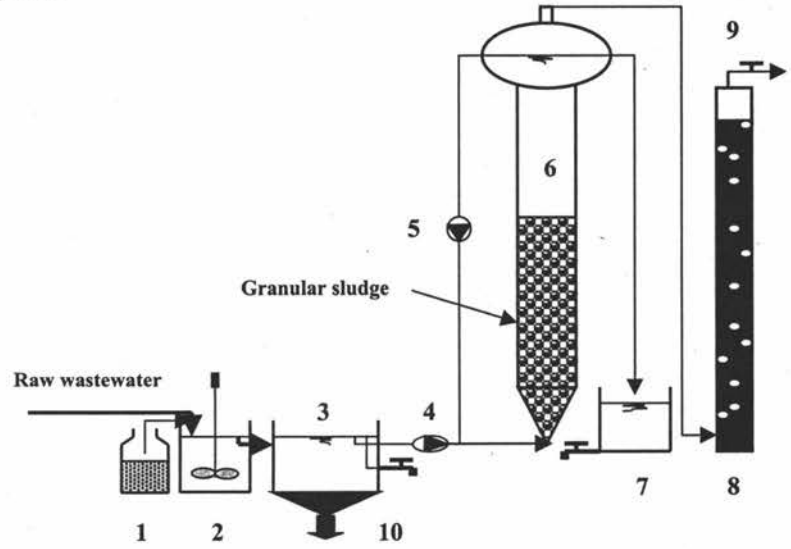


Figure 1. Schematic representation of the experimental set-up (not to scale)

1.Coagulant ($FeCl_3$ or WEMOS), **2**. Mixing tank, **3**. Primary decanter, **4**. Supernatant fuid feeding pump, **5**. Recycling pump, **6**. UASB reactor, **7**. Effluent tank, **8**. Biogas column full with liquid at pH < 4, **9**. Biogas sampling point, **10**. Chemically enriched primary (CEP)-sludge.

The top of the reactor was connected to a gas column. The pH of the liquid into the gas column was lowered to 4, by adding HCl (Vel, Leuven, Belgium) in order to avoid the dissolution of CO_2 of the biogas. Methyl orange (Vel, Leuven, Belgium) was added to the liquid as indicator of the biogas level in the column. The operational parameters of the reactor are given in Table 2.

Table 2. Operational parameters of the UASB reactor

Parameters	Period I ($FeCl_3$)	Period II (WEMOS)
Duration (d)	1 – 82	83 - 170
Volume of the reactor (l)	1.2	1.2
Temperature (°C)	30 ± 1	29 ± 1
Volume of the feed ($l\ d^{-1}$)	12.6 ± 1.4	15.1 ± 1.5
B_v ($g\ CODt\ l^{-1}\ d^{-1}$)	1.4 ± 0.6	4.2 ± 0.4
HRT (h)	2.4 ± 0.3	1.9 ± 0.3
V_{up} ($m\ h^{-1}$)	1.25 ± 0.11	1.3 ± 0.1

Note: B_v = Volumetric loading rate; HRT = Hydraulic retention time; HLR = Hydraulic loading rate; V_{up} = Upflow velocity.

Analytical techniques

Physico-chemical parameters were determined in accordance with the standard methods (APHA, 1992). COD, SS and VSS were determined 2 to 3 times per week. The other parameters in Table 1 were determined once per week. Total coliform (TC), faecal coliform (FC) and faecal streptococci (FS) were enumerated by plating count techniques as described by Kersters *et al.* (1995). Glucose was measured by the glucose oxidase reaction using a biochemical analyser (Model 2500 Select, Biorad).

The CH_4 proportion in the biogas was analysed with an Intersmat IGC 120 MB gas chromatograph connected to Hewlett-Packard 3390 A integrator.

The SMA was determined in batch reactor with grab sample of granular sludge. The test was carried out by the pressure-bottle technique using acetate as substrate in an anaerobic dilution medium (Valcke & Verstraete, 1983).

Organic matter mass balance

The CODt mass balance along the CEPS-UASB system was evaluated by calculating the influent and effluent CODt. Since the COD analyses were not done daily, the average value of two consecutive days of analyses was taken as the value of the intermediate day in which no analyses were done.

The dissolved CH_4 (D_m) in the effluent was estimated as follows (Van Haandel & Lettinga, 1994): $D_m = S_m . P . \eta$
With D_m = COD mass converted to dissolved CH_4 in effluent (mg CODt l^{-1}), S_m = Solubility of CH_4 at atmospheric pressure = 20 mg l^{-1}, P = CH_4 partial pressure in digester (in atmosphere), η = Conversion factor of CH_4 into COD = 4.

RESULTS

Primary sedimentation in jar test

The results of the primary sedimentation test in Tables 3 and 4 show that the CODs/VSS ratio of all the supernatants fluid produced after the CEPS were increased compared to those of the supernatants produced by NPS (0 mg l^{-1} and 0 ml l^{-1}). Moreover, the CODs/VSS ratio of the supernatant fluids from the CEPS increased with the dose of the coagulant. From the dose of 8 ml l^{-1} onwards, the WEMOS gave a higher ratio than the $FeCl_3$. The test with the WEMOS increased the CODs in the supernatant fluid when the dose of the coagulant increased. In contrast, the CODs was partially removed during the test with $FeCl_3$ when the dose increased. The pH and the alkalinity decreased with the increase of the dose of $FeCl_3$ respectively from 7.6 to 6.9 and 404 to 216 mg $CaCO_3$ l^{-1}. The WEMOS less negatively affected these parameters.

The dose of 70 mg l^{-1} and 24 ml l^{-1} were respectively chosen as the optimal dose for the $FeCl_3$ and the WEMOS. These doses did not change negatively the pH and the alkalinity of the wastewater. They appeared acceptable since

they allowed to reach a CODs/VSS ratio of 9 and 16 respectively. Moreover, it was observed that higher doses than 70 mg l^{-1} of $FeCl_3$ can increase the removal of CODs and more than 24 ml l^{-1} of WEMOS can release an excessive external COD. Higher doses would also increase the pre-treatment cost.

Table 3. Physico-chemical characteristics of the raw wastewater and the supernatant fluid, according to the dose of $FeCl_3$, after the jar test

Dose[a]	SV_{60}[b]	pH	Alkali-nity[c]	CODt (mg l^{-1})	CODs (mg l^{-1})	SS (mg l^{-1})	VSS (mg l^{-1})	**CODs/ VSS**
*	+	7.6 ± 0.3	404 ± 9	269 ± 10	140 ± 7	130 ± 10	101 ± 12	**1.4 ± 0.6**
0	1.2 ± 0.2	7.6 ± 0.1	392 ± 5	196 ± 12	134 ± 7	94 ± 11	72 ± 10	**1.9 ± 0.7**
10	3 ± 1	7.6 ± 0.1	323 ± 7	184 ± 10	132 ± 6	54 ± 9	44 ± 6	**3.0 ± 1**
30	8 ±1	7.3 ± 0.2	312 ± 7	160 ± 11	125 ± 7	39 ± 4	31 ± 6	**4.0 ± 1**
50	16 ±2	7.1 ± 0.3	305 ± 6	145 ± 10	120 ± 8	25 ± 5	20 ± 5	**6.0 ± 1**
70	26 ±2	7.1 ± 0.4	300 ± 8	130 ± 9	110 ± 5	15 ± 4	12 ± 5	**9.2 ± 1.1**
90	28 ±2	7.1 ± 0.1	249 ± 5	120 ± 10	105 ± 7	14 ± 4	11 ± 4	**9.5 ± 1.2**
120	35 ±3	6.9 ± 0.2	216 ± 7	118 ± 11	105 ± 6	14 ± 5	11 ± 5	**9.5 ± 1.2**

Note: [a] = mg l^{-1}; [b] = ml l^{-1}; [c] = mg $CaCO_3$ l^{-1}; * = Raw wastewater completely mixed; + = Not determined; SV_{60} = Volume of sludge after one hour settling; 0 mg l^{-1} is relative to supernatant fluid obtained from natural sedimentation.

Table 4. Physico-chemical characteristics of the raw wastewater and the supernatant fluid, according to the dose of WEMOS, after the jar test

Dose[a]	SV_{60}[b]	pH	Alkali-nity[c]	CODt (mg l^{-1})	CODs (mg l^{-1})	SS (mg l^{-1})	VSS (mg l^{-1})	**CODs/ VSS**
*	+	7.6 ± 0.3	404 ± 9	269 ± 10	140 ± 7	130 ± 10	101 ± 12	**1.4 ± 0.6**
0	1.2 ± 0.2	7.6 ± 0.1	392 ± 6	195 ± 12	133 ± 9	93 ± 11	74 ± 12	**1.8 ± 0.6**
0.2	1.2 ± 0.3	7.6 ± 0.2	390 ± 8	194 ± 10	144 ± 9	90 ± 8	72 ± 9	**2.0 ± 1**
2	1.4 ± 0.3	7.4 ± 0.1	390 ± 9	199 ± 10	149 ± 8	49 ± 5	41 ± 6	**3.6 ± 1.3**
8	4.3 ± 0.7	7.5 ± 0.3	385 ± 7	209 ± 13	184 ± 7	28 ± 5	23 ± 4	**8.0 ± 2**
16	9.5 ± 1.1	7.6 ± 0.1	390 ± 7	238 ± 11	213 ± 9	25 ± 4	21 ± 5	**10.2±1.8**
24	10 ± 1	7.6 ± 0.4	390 ± 8	342 ± 10	313 ± 7	24 ± 6	19 ± 5	**16.5±1.4**
32	11 ± 1	7.5 ± 0.2	387 ± 6	427 ± 12	402 ± 8	24 ± 5	19 ± 6	**21.6±1.3**

Note: a = ml l^{-1}; b = ml l^{-1}; c = mg $CaCO_3$ l^{-1}; * = Raw wastewater completely mixed; + = Not determined; SV_{60} = Volume of sludge after one hour settling; 0 ml l-1 is relative to supernatant fluid obtained from natural sedimentation.

Table 5 shows the results of the characterisation of the supernatants fluid from pre-treatment with the optimal doses of $FeCl_3$ and WEMOS. The glucose concentration in the supernatant fluid increases when WEMOS is used for pre-treatment and decreases when $FeCl_3$ is used. Supernatant fluid from pre-treatment with $FeCl_3$ had in general lower concentrations of metals ions than that from pre-treatment with WEMOS.

Table 5. Effect of $FeCl_3$ (70 mg l^{-1}) and WEMOS (24 ml l^{-1}) on glucose and metal ions concentrations of the supernatant fluid

Parameters	Raw wastewater	Supernatant $FeCl_3$	Supernatant WEMOS
CODt (mg l^{-1})	269 ± 10	130 ± 9	342 ± 10
Glucose	17 ± 5	8 ± 2	58 ± 7
Glucose as % CODt	5 - 8	5 - 7	15 - 18
Ca^{2+}	75 ± 4	41 ± 3	48 ± 4
K^{+}	11 ± 2	7 ± 2	9 ± 2
Mg^{2+}	6 ± 2	3 ± 2	5 ± 3
Na^{+}	98 ± 7	55 ± 5	65 ± 7
Fe^{2+}	1.9 ± 0.2	2.1 ± 0.3	0.35 ± 0.01
Zn^{2+}	1.5 ± 0.3	0.26 ± 0.4	0.3 ± 0.02
Ni^{+}	0.08 ± 0.02	0.06 ± 0.02	0.06 ± 0.01

Continuous operation of the CEPS

Period 1

During period 1, the CEPS was carried out with $FeCl_3$. The average results are summarised in Table 6. The addition of the $FeCl_3$ removed 50% of the CODt in the form of precipitated sludge. The CODs removal was 24%. The CODt and the CODs of the supernatant fluid were 134 and 102 mg l^{-1} respectively. The sludge (not thickened) production was on average 2.8% of the total volume of the raw wastewater. Its concentration was on average 4.7 g CODt l^{-1}. The SS and the VSS were respectively removed at 75% and 79%. The removal of SS increased the CODs/VSS ratio from 1.4 to 5. The ratio fluctuated between 4 and 8 (Figure 2, PI). Compared to the NPS, the CEPS with $FeCl_3$ increased the CODs/VSS ratio by a factor 3. During the CEPS, 15% of nitrogen and 60% of phosphorus were removed. On average, the pH and the alkalinity dropped from 7.6 to 7.3 and 361 to 305 mg $CaCO_3$ l^{-1}

respectively. During the treatment, the iron was slightly released in the supernatant fluid.

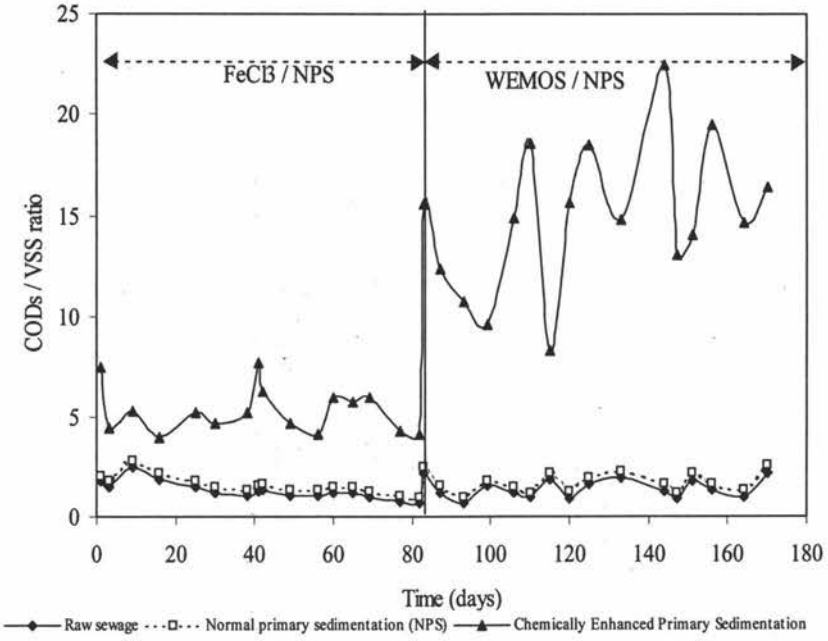


Figure 2. CODs/VSS ratio evolution in the raw wastewater, the supernatants fluid from the natural (NPS) and the chemical (CEPS) sedimentation (with $FeCl_3$ and WEMOS) as a function of time

Table 6. Removal efficiencies (%) of the CEPS-UASB system during the two periods of the experiment

Parameters		$FeCl_3$	UASB*	Total removal	WEMOS	UASB*	Total removal
CODt	($mg\ l^{-1}$)	50 ± 8	54 ± 4	77 ± 3	(42 ± 4)	71 ± 9	63 ± 12 (83 ± 5)
CODs	($mg\ l^{-1}$)	24 ± 12	55 ± 5	66 ± 7	(32 ± 3)	72 ± 10	33 ± 23 (81 ± 6)
SS	($mg\ l^{-1}$)	75 ± 10	51 ± 14	88 ± 4	77 ± 9	56 ± 8	90 ± 4
VSS	($mg\ l^{-1}$)	79 ± 7	47 ± 17	89 ± 4	80 ± 4	56 ± 9	91 ± 2
TKN	($mg\ l^{-1}$)	15 ± 9	10 ± 3	24 ± 8	(+)	11 ± 2	(+)
NH_4^+-N	($mg\ l^{-1}$)	5 ± 2	4 ± 1	10 ± 3	(+)	6 ± 3	(+)
Ptotal	($mg\ l^{-1}$)	60 ± 6	18 ± 8	67 ± 6	(+)	22 ± 2	(+)
PO_4^{3-}-P	($mg\ l^{-1}$)	79 ± 9	(++)	76 ± 10	(+)	(++)	(+)

Note: *= Removal efficiency calculated on the basis of supernatant fluid COD and effluent COD; ( ) = The removal efficiencies between bracket were calculated by considering the increase of COD, in the influent, due to the WEMOS; (+) = Values increased due to the WEMOS; (++) = Value increased due to the mineralization of the phosphorus.

Period 2

The results of the CEPS carried out with the WEMOS are presented in Table 6. The addition of the WEMOS removed 77% of the SS and 80% of the VSS. However, the CODt and CODs of the supernatant increased from 268 mg l^{-1} to 336 mg l^{-1} and from 139 mg l^{-1} to 305 mg l^{-1} respectively. Taking into account the COD released by the WEMOS (addition of 24 ml l^{-1} induced on average 312 mg COD l^{-1}) the CODt removal was 42% and the CODs removal was 32%. The sludge (not thickened) production was 1% of the volume of the raw wastewater. Its concentration was on average 18.6 g CODt l^{-1}. The supernatant fluid had on average a CODs/VSS ratio of 15. The ratio fluctuated between 8 and 23 (Figure 2, PII). The average ratio of 15 was 10 and 3 times higher than that of the NPS and the CEPS with $FeCl_3$ respectively. The CEPS with the WEMOS increased the nitrogen and the phosphorus content of the supernatant fluid with 24% and 20% respectively.

UASB reactor performance

Period 1

During the first period, from day 1 to 40, the reactor was fed daily with 10 l of wastewater. The B_v of the reactor was on average 1.2 g CODt l^{-1} d^{-1} and the HRT was on average 3 hours. From day 41 to 82 the feed flow was increased to 15 l d^{-1}. This resulted in a B_v of 1.5 g CODt l^{-1} d^{-1} and a HRT of 2 hours.

Figures 3a and 3b (PI) show respectively the change in CODt and CODs concentrations. The quality of the effluent of the UASB reactor remained stable for both parameters during the whole experiment while the B_v increased and the HRT decreased from day 41 to 82. The average CODt of the effluent was 61 mg l^{-1}. The reactor showed also a good stability in removal of the VSS, with an average VSS concentration of 11 mg l^{-1}. On average, the removal efficiency of the reactor was 54% for the CODt, 55% for the CODs, 51% for the SS and 47% for the VSS. The reactor produced a very low amount of biogas (28 ml d^{-1}) with CH_4 content of 65%. At the end of the experiment, the SMA of the biomass was 0.20 ± 0.03 gCH_4-COD g^{-1}VSS d^{-1}.

The mass balance of the CEPS-UASB system is represented in Figure 4a. It shows that 3.484 g CODt were daily treated in the CEPS step. 1.751 g CODt d^{-1} were treated in the UASB reactor. The digestion of this fraction resulted in 0.769 g CODt d^{-1} leaving with the effluent. 0.12 g CODt d^{-1} was converted into excess sludge and 0.708 g CODt d^{-1} was converted into CH_4. However, only 0.053 g CODt d^{-1} was released from the liquid phase and 0.655 g CODt d^{-1} left the reactor with the effluent.

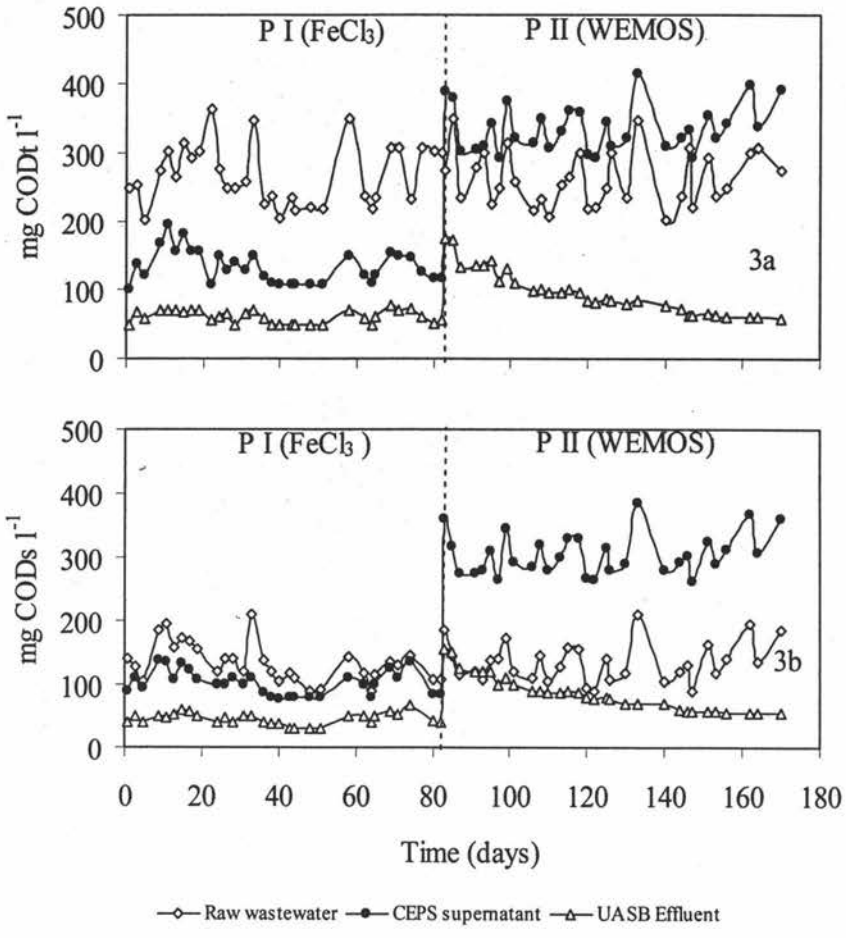


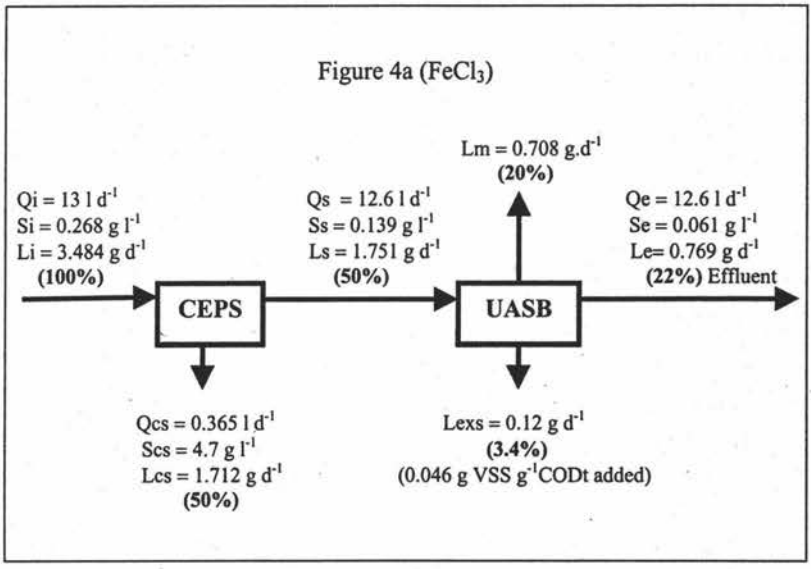
Figure 3. COD variations of the raw wastewater, the supernatant fluid from the CEPS and the effluent from the UASB reactor as a function of time

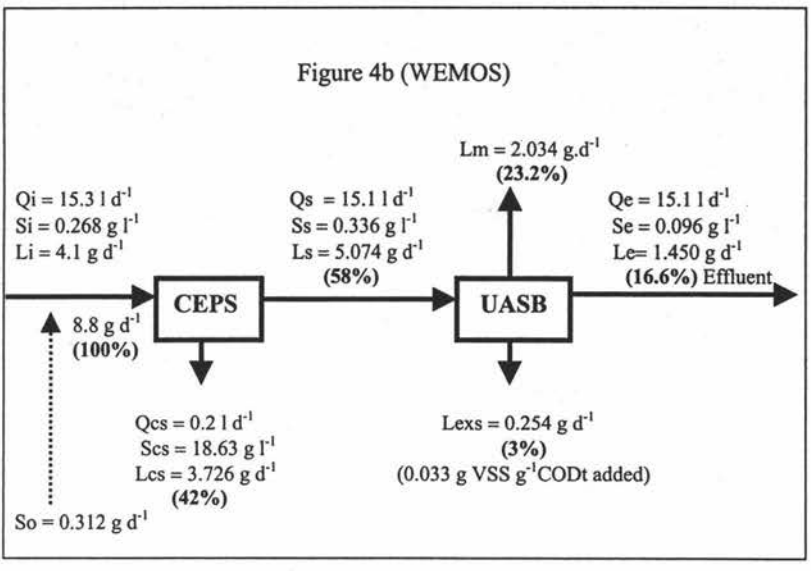
Period 2

During the second period, the flow of the feed was maintained at 15 l per day. As a consequence, the HRT remained at 2 hours. As the WEMOS increased the COD in the influent (supernatant fluid), the average B_v increased to 4 g CODt l^{-1} d^{-1}.

Figures 3a and 3b (PII) show respectively the change in CODt and CODs concentrations. During the first week of this period, the effluent quality of the UASB reactor decrease since the CODt concentration increased till 170 mg l^{-1}. Then the quality gradually improved and reached the level of period 1 indicating a good adaptation of the anaerobic biomass to the exogenous carbon source released by the WEMOS. At the end of the second period, the CODt in the effluent was 59 mg l^{-1}. Yet, the average CODt was 96 mg l^{-1}. VSS concentration remained at a low level (9 mgVSS l^{-1}). On average, the removal efficiency of the reactor was 71% for the CODt, 72% for the CODs, 56% for the SS and 56% for the VSS. The gas production was on average 594 ml d^{-1} with a CH_4 content of 70%. At the end of the second period, the SMA of the biomass was 0.25 ± 0.02 gCH_4-COD g^{-1}VSS d^{-1}.

The mass balance of the CEPS-UASB system is represented in Figure 4b. It shows that 8.8 g CODt were daily treated in the CEPS step. 5.074 g CODt d^{-1} were treated in the UASB reactor. The digestion of this fraction resulted in 1.450 g CODt d^{-1} leaving with the effluent. 0.254 g CODt d^{-1} was converted into excess sludge and 2.034 g CODt d^{-1} was converted into CH_4. However, only 1.188 g CODt d^{-1} was released from the liquid phase and 0.846 g CODt d^{-1} left the reactor with the effluent.





Q = Flow, S = Concentration in CODt, So = Extra COD of the WEMOS, L = CODt load, Lexs = CODt load in the form of excess sludge, Lm = CODt load converted in CH_4, i = Influent, s = Supernatant fluid, e = Effluent, cs = Chemical sludge.

Figure 4. Mass balances of CEPS-UASB treatment

Hygienic performance of the CEPS-UASB system

Table 8 shows the average concentration of TC, FC and FS in the supernatants fluid and the effluents of the UASB reactor for both periods of operation. These concentrations are compared to the concentrations of the raw wastewater. The CEPS with $FeCl_3$ and WEMOS have a fair effect on the removal of bacteria. The UASB reactor has rather low removal efficiency.

Table 8. Concentration of TC, FC and FS in the different stages of the CEPS-UASB system compared to the raw wastewater

Parameter[a]	Raw wastewater	CEPS-$FeCl_3$	UASB reactor	CEPS-WEMOS	UASB reactor
TC	8.0 ± 0.2	5.8 ± 0.3	5.4 ± 0.3	5.5 ± 0.1	5.2 ± 0.2
FC	7.4 ± 0.4	4.5 ± 0.4	3.6 ± 0.3	4.9 ± 0.3	4.1 ± 0.1
FS	6.6 ± 0.2	3.8 ± 0.2	3.3 ± 0.2	4.2 ± 0.2	3.5 ± 0.3

Note: [a] = Log cfu 100 ml^{-1}

DISCUSSION

Wastewater characteristics and CEPS operation

During the experimental period of 170 days, the raw wastewater used had on average a CODt content of 268 mg l^{-1}. It can therefore be classified as a weak domestic wastewater (Tchobanoglous & Burton, 1991). This COD value was two times lower than values already reported for the same treatment plant (Chen, 1996; Ghyoot & Verstraete, 1997). This was due to the intensive rain during the experimental period since in Gent, the sewers for domestic wastewater and rain water collection are the same.

During the CEPS with both coagulants, the CODs/VSS ratio fluctuated significantly (Figure 2). The ratio's varied stronger than the result obtained in the jar tests. The CODs/VSS ratio depended on the amount of CODs initially available in the wastewater. A lower CODs generally leads to a low CODs/VSS ratio. The ratio also depended on the type of coagulant used.

In case of the $FeCl_3$, in parallel to the SS removal, also part of the CODs was removed. This contributed to a decrease in the CODs/VSS ratio of the wastewaters containing low CODs. This explains why the average value obtained for the continuous CEPS was 5 while the result of the jar test was 9. The value observed in continuous CEPS represented only half of the value suggested to achieve suitable treatment in UASB reactor (De Baere & Verstraete, 1982; De Baere *et al.*, 1984). However, since the VSS content of the supernatant fluid was low (20 mg l^{-1} on average), this value could be considered as acceptable. While 15% of the nitrogen and 60% of the phosphorus were removed during the CEPS, sufficient amounts of these compounds remained in the supernatant fluid. Thus, an equilibrium COD/N/P higher than the minimum of 100/1.25/0.25 necessary for anaerobic digestion (Thaveesri, 1995) was still present in the supernatant fluid. Indeed the ratio was 100/23/2.

The COD released by the WEMOS contributed to increase the CODs/VSS ratio of the supernatant fluid. Since the amount of COD added by the WEMOS was constant, the wastewater with low CODs content had a low CODs/VSS ratio compared to the wastewater with high CODs. This led to the fluctuation observed in Figure 2 (PII). Both $FeCl_3$ and WEMOS showed a similar removal efficiency of VSS. The higher CODs/VSS ratio observed with the WEMOS was due to the COD released by this coagulant in the supernatant fluid. The increase of COD and nutrients during wastewater pre-treatment with the WEMOS has also been observed in other works (Ndabigengesere & Narasiah, 1998b). The equilibrium COD/N/P of 100/14/3 of the supernatant fluid was not detrimental to subsequent anaerobic digestion.

During the experiment, the WEMOS produced 2.8 times less sludge (in terms of volume) than the $FeCl_3$. The low sludge production with the WEMOS compared to the synthetic coagulants have been reported in several works (Ndabigengesere *et al.*, 1995; NDabigengesere & Narasiah, 1998a; NDabigengesere & Narasiah, 1998b).

UASB reactor

The COD removal efficiencies obtained in both periods of operation of the UASB reactor are comparable to the results reported for several UASB reactors treating low strength domestic wastewater at a temperature above 20 °C, as indicated in Table 7. However, these former results were obtained at a HRT varying in the range of 4 to 12 hours. They were probably due to interception of the SS in the reactor.

The UASB reactor was operated during 82 days (period 1) and 88 days (period 2) without any necessity to remove the excess of biomass while the formation of fluffy biomass was noticed. In classical UASB reactor treating raw domestic wastewater, once or twice a week, the excess of sludge has to be discharged in order to control the sludge hold up effect on the effluent quality (Lettinga *et al.*, 1993).

Despite the formation of the fluffy sludge, the reactor effluent quality was not affected. The results demonstrate that, by coupling the CEPS to the UASB reactor, the time necessary for the reactor to reach its maximum sludge hold-up can be considerably increased.

The excess sludge produced in the UASB reactor was 0.046 gVSS g^{-1}CODt added (period 1) and 0.033 gVSS g^{-1}CODt added (period 2). These values are significantly lower than the excess sludge production of 0.1 - 0.18 gVSS g^{-1}CODt added commonly found in classical anaerobic systems (Van Haandel & Lettinga, 1994). Since the influent was free of the most important fraction of SS it can, based on the present result, be postulated that the excess sludge produced was generated mainly biologically. Indeed, during raw domestic wastewater treatment in a UASB reactor, an important fraction of excess sludge is due to interception in the sludge bed of poorly biodegradable solids present in the influent (Van Haandel & Lettinga, 1994).

The low biogas production in period 1 was foreseeable since the influent COD concentration after the CEPS was low (134 mg l^{-1}). The anaerobic treatment

of such a low strength wastewater generally leads to the loss of more than 50% of CH_4 produced via the effluent leaving the reactor (Lettinga *et al.*, 1993). This could be confirmed by the SMA of the granular sludge, which was 0.20 ± 0.03 g CH_4-COD g^{-1} d^{-1} at the end of period 1. This value, compared to the initial activity of 0.21 ± 0.02 g CH_4-COD g^{-1} d^{-1}, demonstrates that the methanogenic bacteria were active during the treatment.

Table 7. Anaerobic treatment of low strength domestic wastewater in UASB reactor at temperature ≥ 20 °C

Temperature (°C)	HRT (h)	Influent concentration (mg l^{-1})			Removal efficiency (%)			Authors
		CODt	CODs	SS	CODt	CODs	SS	
20	12	330	144	+	68	52	75	Lettinga *et al.* (1983)
24 - 26	4 - 8	267	+	215	65	+	70	Schellinkhout *et al.* (1985)
21 - 25	4.7	265	+	123	50	+	56	(Vieira, 1988)
21 - 25	9	316	+	170	70	+	73	(Vieira, 1988)
30	7	300	120	180	77	58	75	Agrawal *et al.* (1997)
30	2.4	134	102	31	54	55	51	This work
29	1.9	336	305	30	71	72	56	This work

Note: + = Not indicated.

At the end of the second period, the activity had increased to 0.25 ± 0.02 g CH_4-COD g^{-1} d^{-1}. This was higher than the activities of 0.1 (Lettinga *et al.*, 1983), 0.14 – 0.17 (Barbosa & Sant'Anna, 1989) and 0.06 - 0.2 gCH_4-COD g^{-1} d^{-1} (Draiijer *et al.*, 1992) reported for anaerobic biomass treating raw domestic wastewater in UASB reactor. The increase of the activity in the second period was likely due to the increase of carbohydrate in the supernatant from pre-treatment with WEMOS (Table 5). Carbohydrates are known to be the most suitable substrate for the acidogenic bacteria, which are essential for the growth of the methanogens (Thavesri, 1995). Besides that, the greater availability of metals ions in the supernatant from pre-treatment with WEMOS (Table 5) might also play a significant role in stimulating the activity of the methanogens (Champiat, 1994).

Total treatment

In the present work, the CEPS allowed the UASB reactor to run at a HRT of 2 hours with a good quality of the effluent. This is very interesting since it significantly decreases the reactor volume necessary to treat, in a classical one step UASB reactor, the same raw wastewater (in terms of flow and CODt content) with the same final effluent quality.

A raw domestic wastewater with a CODt of 268 mg l^{-1} and a final effluent with a CODt of 61 mg l^{-1} (effluent of period 1), means that 77% removal was achieved. According to the model of Van Haandel *et al.* (1996) to reach this performance, the reactor should operate at least at a HRT of 5 hours. Hence, by coupling the CEPS to the UASB reactor, the volume needed to get the same effluent quality can be decreased by a factor 0.6. Taking into account the volume of the primary decanter designed with a terminal velocity (V_t) of 0.8 m h^{-1} and a HRT of 1 hour, the total volume decrease can be a factor 0.4. Therefore, the implementation of a CEPS can yield economic benefits in terms of the reactor investment costs. The construction cost of a UASB reactor is in the range of 181 to 300 EURO m^{-3} depending on the material used and the country (Schellinkhout & Collazos, 1992; Vieira & Sousa 1986).

The volume decrease by a factor 0.4 should lead to a gain of 72 to 120 EURO per m^3 of reactor. Since 1 kg of $FeCl_3$ costs 0.46 EURO, the 70 mg l^{-1} dosed costs 0.03 EURO per cubic meter. This extra cost seems quite acceptable. 1 kg of *M. oleifera* seeds costs 36 EURO, which is 80 times more expensive than the $FeCl_3$. Considering the 24 ml l^{-1} used the treatment cost with the WEMOS correspond to 43 EURO per cubic meter. It should be however indicated that the cost reported here is the selling cost of the seeds for experimental purposes. Many research teams worldwide are indeed currently busy in studying the use of *Moringa oleifera* seeds in water treatment. For better comparison, one should normally include in the cost evaluation the environmental impact of both coagulants. WEMOS has the advantage to be biodegradable, it is therefore environmentally friendly. Finally, The cost of the seeds can be lowered to a reasonable level if an extensive culture of *Moringa oleifera* is promoted worldwide.

The evaluation of the CEPS-UASB system mass balance showed that 4.6% and 15% of the COD were not accounted for in the balance of period 1 and period 2 respectively. This is related to the difficulty of estimating the total amount of the COD supplied to the reactor. In fact, sampling and COD analyses were not done daily but 2 to 3 times per week during the experiment. The difficulty of making a COD balance has been reported by other researchers (De Sousa & Foresti, 1996; Lettinga *et al.*, 1983). The former (De Sousa & Foresti, 1996) reported a deficit of 30%, which is 2 to 6 times higher than that observed in this work. To have a perfect mass balance (100%) one needs daily analyses of the COD of the influent, the effluent and the biogas. This is however costly and time consuming. The current 85 to 95% balance therefore appears acceptable.

The CEPS-UASB system showed a fair hygienic performance. However, in terms of microbiology, the final effluent is unsuitable as such for irrigation of crops likely to be eaten uncooked since it does not meet the World Health Organisation (1989) criteria of TC and FC < 3 Log cfu 100 ml^{-1}.

CONCLUSIONS

The purpose of this work was to study the technical feasibility of the CEPS-UASB system for the treatment of domestic wastewater. Both $FeCl_3$ and WEMOS were effective to remove the SS from the raw wastewater. Furthermore, the application of WEMOS increased the CODs/VSS ratio from 1.4 to 15 (3 and 11 times higher than that of the $FeCl_3$ and the NPS respectively) by contributing to the COD content of the supernatant, thereby enhancing the performance of the reactor.

The overall results indicate that the CEPS allows 1°) the treatment of domestic wastewater in a UASB reactor at a short HRT, 2°) to prevent the negative effect of the SS on the activity of the methanogens, 3°) to increase the maximum sludge hold up time of the reactor.

The WEMOS increases the nutrient concentration in the final effluent. The use of WEMOS should be interesting in case the effluent of the UASB reactor has to be reused on farmland deficient in nitrogen and phosphorus. A strong disinfection step is therefore necessary to meet the international standards of water reuse in agriculture. $FeCl_3$ should be advised in case the UASB effluent will be discharged in river.

At the end of this work, it is still a question of how the sludge produced from the CEPS step can be managed.

ACKNOWLEDGMENTS

This work was partially financed by the Government of Côte d'Ivoire through a grant. The authors are grateful to Mr Doulaye Kone for sending them the seeds of *M. oleifera*.

CHAPTER VII

ANAEROBIC CO-DIGESTION OF VFG-WASTE AND CEP-SLUDGE WITHOUT ADDITION OF EXTERNAL ALKALINITY

Kalogo Y. & Verstraete W.

A modified version of this text is submitted for publication as:

Kalogo Y. Biey M.E. & Verstraete W. Anaerobic co-digestion of VFG-waste and CEP-sludge without addition of external alkalinity. *Journal of Chemical Technology and Biotechnology*

ANAEROBIC CO-DIGESTION OF VFG-WASTE AND CEP-SLUDGE WITHOUT ADDITION OF EXTERNAL ALKALINITY

ABSTRACT - Anaerobic co-digestion of vegetable fruit and garden (VFG)-waste and chemically enriched primary (CEP)-sludge from synthetic ($FeCl_3$) and natural (WEMOS) coagulant treatment was investigated in lab-scale batch reactors. The experiment was performed at mesophilic conditions (33 °C) with the ratio CEP-Sludge:VFG-waste (%) of 100 : 0; 75 : 25; 50 : 50; 25 : 75. A positive control 0 : 100 receiving 11.4 g $NaHCO_3$ l^{-1} and a negative one receiving no buffering agent were also studied. Each reactor received in total 50 ml of feed. Both VFG-waste and CEP-sludge had a concentration of 5% (w/v) total solids (TS). In the reactor treating only VFG-waste (negative control), inhibition occurred due to the accumulation of up to 5 g l^{-1} of volatile fatty acids (VFA). As a consequence, there was a significant decrease in the pH and the reactor performance. All the reactors fed with VFG-waste and CEP-sludge showed good production of CH_4 and volatile solids (VS) destruction efficiency between 50 to 69%. The high buffering capacity contained in the CEP-sludge served to avoid the pH decrease, thereby stabilising the system. Overall results indicate that anaerobic digestion of VFG-waste is compatible with that of CEP-sludge. Furthermore, the CEP-sludge makes the anaerobic breakdown of VFG-waste possible without addition of extra alkalinity, e.g. $NaHCO_3$, for pH stabilisation. This can therefore yield some economics benefits.

Keywords: Alkalinity, Anaerobic digestion, CEP-sludge, co-digestion, VFG-waste

INTRODUCTION

Anaerobic digestion is currently one of the most widely used techniques for the treatment of organic solid waste. The interest of this technique is mainly related to the fact that it offers the possibility to recover energy, which can be used for heating and/or electricity production. Within the last fifteen years, many anaerobic reactors such as the Dranco (De Baere & Verstraete, 1984),

the Valorga (St-Joly, 1992) and the Biomet (Szikrist *et al.*, 1988) have been developed for the treatment of municipal solids waste (MSW). However, anaerobic digestion of MSW was shown to be unstable especially at high feed concentrations (Borzaconi *et al.*, 1997). During the treatment, the rate of volatile fatty acid (VFA) production can be higher than the rate of VFA conversion to CH_4. This generally occurs in single stage reactors, resulting in a concomitant pH drop, cessation of CH_4 generation, and ultimate process failure (Ghosh, 1997). Low solids anaerobic digestion can also show signs of failure as the pH can drop below 6.5 when the chemical oxygen demand (COD) loading rate becomes higher than 6 g l^{-1} d^{-1} (Waweru, 1997). To overcome the inhibition of the process, sufficient alkalinity has to be present in the digester (Tchobanoglous *et al.*, 1993). Generally, products such as $NaHCO_3$ or $Ca(OH)_2$ are used to adjust the alkalinity of anaerobic reactors (Ten Brummeler *et al.*, 1988). However, the addition of external chemicals can greatly increase the treatment cost.

Chemically enhanced primary sedimentation is a process consisting of the addition of chemical coagulants (aluminium, iron chloride…) to wastewater in order to achieve a better removal of suspended solids (SS) or precipitation of phosphorus (P). This is successfully used in many countries such as the United States, Hong Kong, France, Norway, Sweden and Spain for the treatment of domestic wastewater (Karlson, 1985; Harleman *et al.*, 1991; Shao *et al.*, 1993; Chu *et al.*, 1998). Although the coagulants improve the effluent quality, they lead to a large amount of chemically enriched primary (CEP)-sludge characterised by a high nutrient content (Chu *et al.*, 1998). This large amount of waste can not be discharged without further treatment. Ghyoot & Verstraete (1997) have investigated the anaerobic degradation of CEP-sludge. These authors indicated that the treatment was feasible without the need to supply extra alkalinity. This was explained by the increase of NH_4^+-N during the treatment. Indeed, as ammonia is set free during the breakdown of proteins, there is a build up of NH_4HCO_3 which serves to buffer the digester content (De Baere *et al.*, 1982).

Co-digestion is a method used to treat different types of organic wastes together. This method has become an active field of research since it offers many advantages compared to separate treatment. It can be applied to dilute or concentrate organic waste at an appropriated dry matter content (Oleszkiewicz & Poggi-Varaldo, 1997), reduce the treatment cost per volume of reactor (Nordberg & Edström, 1997), improve the nutrient balance of the main substrate (Ghosh *et al.*, 1980), increase the specific biogas productivity of the reactor (Weiland, 1997) or prevent the toxicity of inhibitors (Mathisen, 1997). The feasibility of anaerobic co-digestion of MSW and waste activated sludge has already been investigated in batch reactors operated mesophilicaly and thermophilicaly (Hamzawi *et al.*, 1998; Oleszkiewicz & Poggi-Varaldo, 1997). In the present work, the anaerobic co-digestion of vegetable fruit and garden (VFG)-waste and CEP-sludge was investigated in batch reactors. The study focused on the potential application of CEP-sludge, from domestic wastewater pre-treatment, to improve the buffer capacity of anaerobic reactors treating VFG-waste. The effect of digestion time on the performance of the reactors was also examined.

MATERIALS AND METHODS

Organic solid waste

The VFG-waste used in this experiment was obtained from the municipal solid waste treatment plant of the city of Antwerp (Belgium). Prior to use, the waste was diluted with distillate water to the desired total solid (TS) concentration.

Two types of CEP-sludge were used in the study. They were obtained from pre-treatment of domestic sewage with respectively 70 mg l^{-1} of synthetic coagulant ($FeCl_3$) or 24 ml l^{-1} of water extract of *Moringa oleifera* seeds (WEMOS), a natural coagulant. The CEP-sludge was produced as described in chapter VI.

Inoculum

The inoculum was a mixture of anaerobic digested VFG-waste obtained from the municipal solid waste treatment plant of the city of Antwerp and anaerobic digested sewage sludge obtained from Zele (Belgium) sewage sludge treatment plant. Before use, the inoculum was mixed at the ratio of 50%:50% (v/v) and reactivated in mesophilic condition (33 °C) during one month with a mixture of, in terms of COD, 50% acetate (Vel, Leuven, Belgium), 18% butyrate (Acros, Geel, Belgium), 7% propionate (Acros, Geel, Belgium) and 25% Sucrose (Aldrich, Milwaukee, USA). At the end of the reactivation period the inoculum had a specific methanogenic activity of 0.6 g COD-CH_4 g^{-1}VSS d^{-1} .

Experimental procedure

The whole experiment was carried out in batch with serum bottles as reactors. The reactors had a total volume of 120 ml. The co-digestion of VFG-waste, 5% (w/v) TS, with CEP-sludge, 5% (w/v) TS, was investigated at different ratio CEP-sludge:VFG-waste ratio's under the same load (50 g TS l^{-1} reactor). In the first part of the experiment the application of CEPS to stabilise the buffer capacity of the anaerobic reactors treating VFG-waste was investigated. This experiment was operated for 20 days. The CEP-sludge:VFG-waste ratio (%) of 100 : 0; 75 : 25; 50 : 50; 25 : 75 were studied. A positive control 0:100 receiving 11.4 g $NaHCO_3$ l^{-1} and a negative one receiving no buffering agent were also studied. Each reactor received in total 50 ml of feed. The $NaHCO_3$ was purchased from Vel, Leuven, Belgium.

In the second part of the experiment the effect of digestion time on the performance of the reactors was examined with the reactors containing the VFG-waste:CEP-sludge ratio (%) of 75 : 25; 50 : 50 and 25 : 75. Four series of reactors were operated respectively at digestion times of 15, 20, 30 and 40 days.

The characteristics of the waste fed to the reactors are summarised in Table1. The reactors were triplicate and inoculated with the ratio inoculum/substrate solid ratio of 1/3. The reactors were closed with a black rubber septum and saddled with an aluminium screw cap. Subsequently, the headspace of the reactors was flushed for two minutes with nitrogen gas, to remove the oxygen. The reactors were operated in mesophilic conditions (33 ± 2 °C).

Analytical techniques

Physico-chemical parameters including are ammonia nitrogen (NH_4^+-N), organic carbon (C), COD, heavy metals, pH, P, total Kjedahl nitrogen (TKN), TS, VFA and volatile solids (VS) were determined in accordance with the standard methods (APHA, 1992). Bicarbonate alkalinity (BA) and total alkalinity (TA) were determined by acid titration from the initial pH of the samples to pH 5.75 and 4.5 respectively (APHA, 1992). The biogas production was evaluated from pressure measurement with a tensimeter (SMS, Arizona, USA). CH_4 proportion in the biogas was analysed with an Intersmat IGC 120 MB gas chromatograph connected to Hewlett-Packard 3390 A integrator.

Calculation

To calculate the sum of VFAs concentration (C) in terms of acetic acid (HAc) we divided each VFA concentration (e.g. acetic, propionic, butyric, valeric acid) by its molecular weight and multiply by the molecular weight of acetic acid as follows:

$$C_{VFA\text{-}HAc} = C_{HAC} + C_{Propionic}.60/74 + C_{Butyric}.60/88 + C_{Valeric}.60/102$$

Table 1. Characteristics of the substrates fed to the different reactors

Parameters	RF_0	RF_{25}	RF_{50}	RF_{75}	RW_0	RW_{25}	RW_{50}	RW_{75}	RNC	RPC
pH	7.1 (0.1)	7.1 (0.2)	7.0 (0.1)	7.0 (0.1)	7.3 (0.2)	7.2 (0.1)	7.2 (0.3)	7.1 (0.1)	6.8 (0.1)	7.5 (0.3)
BA ($g\ CaCO_3\ l^{-1}$)	1.9 (0.4)	1.3 (0.2)	1.0 (0.3)	0.5 (0.1)	1.9 (0.5)	1.7 (0.3)	1.6 (0.4)	0.5 (0.1)	0.3 (0.1)	4.5 (0.8)
TA ($g\ CaCO_3\ l^{-1}$)	3.8 (0.8)	3.2 (0.3)	2.4 (0.4)	1.2 (0.1)	3.4 (0.3)	3.2 (0.3)	2.9 (0.4)	1.2 (0.2)	1.1 (0.1)	5.4 (0.8)
TKN ($g\ l^{-1}$)	1.3 (0.2)	1.2 (0.3)	1.0 (0.1)	1.0 (0.2)	1.5 (0.3)	1.4 (0.2)	1.4 (0.1)	1.1 (0.1)	0.9 (0.2)	0.9 (0.2)
NH_4^+-N ($mg\ l^{-1}$)	53 (6)	51 (4)	59 (7)	57 (4)	40 (5)	45 (6)	51 (5)	54 (5)	58 (9)	58 (9)
P ($g\ l^{-1}$)	0.84 (0.01)	0.78 (0.02)	0.42 (0.02)	0.36 (0.03)	0.09 (0.01)	0.19 (0.02)	0.24 (0.01)	0.3 (0.01)	0.32 (0.03)	0.32 (0.03)
COD ($g\ l^{-1}$)	24.1 (0.8)	32.5 (0.3)	42.5 (0.5)	54.9 (0.2)	59.5 (0.5)	59.2 (0.4)	60.0 (0.3)	58.5 (0.4)	68.8 (0.3)	68.8 (0.3)
TS ($g\ l^{-1}$)	45.7 (0.5)	46.1 (0.3)	48.7 (0.3)	49.1 (0.2)	45.1 (0.2)	46.8 (0.5)	47.4 (0.3)	48.6 (0.4)	49.8 (0.2)	49.8 (0.2)
VS ($g\ l^{-1}$)	21.1 (0.3)	25.1 (0.5)	31.9 (0.6)	34.2 (0.3)	37.7 (0.2)	36.8 (0.6)	35.6 (0.4)	34 (0.2)	36.2 (0.5)	36.2 (0.5)
VFA-HAc ($g\ l^{-1}$)	0.28 (0.03)	0.5 (0.01)	0.57 (0.02)	0.58 (0.02)	0.3 (0.03)	0.4 (0.02)	0.52 (0.01)	0.6 (0.02)	0.61 (0.03)	0.61 (0.03)
C ($g\ l^{-1}$)	11	13	16	17	18	19	19	20	21	21

Note: RF and RW are related to reactors containing CEP-sludge from $FeCl_3$ and WEMOS treatment respectively (the subscript indicates the percentage of VFG-waste); RNC = Negative control; RPC = Positive control; ( ) = Standard deviation.

Table 1 (continuation). Characteristics of the substrates fed to the different reactors

Parameters	RF_0	RF_{25}	RF_{50}	RF_{75}	RW_0	RW_{25}	RW_{50}	RW_{75}	RNC	RPC
COD / VS	1.1	1.3	1.3	1.6	1.6	1.6	1.7	1.7	1.9	1.9
C / N	8	11	16	22	12	14	15	16	31	31
TA / TS	0.08	0.07	0.05	0.03	0.08	0.07	0.06	0.03	0.02	0.11
K ($g\ kg^{-1}$ TS)	1.6	3.1	4.5	5.1	0.49	1.59	3.1	3.8	6.1	6.1
Fe ($g\ kg^{-1}$ TS)	113	86	53	22	22	22	16	8	5	5
Zn[a]	802	553	465	257	621	510	414	230	226	226
Cu[a]	135	91	62	38	98	88	90	40	34	34
Ni[a]	61	44	26	20	40	29	15	11	7	7
Cr[a]	16	9	8	8	11	9	9	8	7	7

Note: RF and RW are related to reactors containing CEP-sludge from $FeCl_3$ and WEMOS treatment respectively (the subscript indicates the percentage of VFG-waste); RNC = Negative control; RPC = Positive control; [a] = Expressed in mg kg^{-1} TS.

RESULTS

Addition of CEP-sludge and $NaHCO_3$ on the waste characteristics

As indicated in Table 1, the CEP-sludge from $FeCl_3$ and WEMOS treatment contained more than 3 times more buffer capacity than the raw VFG-waste. Combining CEP-sludge with VFG-waste resulted in an increase of the buffer capacity of the waste samples. As a consequence, the initial buffer/substrate solid ratio of the reactors increased. The mixing also increased the nitrogen content of the reactors. This led to the decrease of the C/N ratio of the reactors.

Direct addition of $NaHCO_3$ to the positive control reactor increased its buffer capacity. This resulted also in an increase of initial buffer/substrate solid ratio of that reactor. All the reactors had an initial pH around the neutral value.

Combining CEP-sludge and VFG-waste modified the mineral composition of the samples. Both CEP-sludge from $FeCl_3$ and WEMOS pre-treatment had a higher content of heavy metals than the raw VFG-waste. The level of heavy metals in the mixtures of VFG-waste and CEP-sludge increased with the increase of the sludge dose (Table 1).

Anaerobic digestion

Effect of the addition of CEP-sludge on the performance of the reactors

The anaerobic digestion of the samples was daily monitored during 20 days. Figure 1 and Figure 2 indicate the net cumulative CH_4 production during the experimental period. The CEP-sludge addition reactors and the positive control reactor showed a good biogas production with CH_4 content varying between 68 to 75%. The negative control reactor had low CH_4 content. After 4 days of operation, almost no CH_4 could be detected in that reactor. The biogas had 98-100% carbon dioxide (CO_2) and as a consequence, there was a low cumulative CH_4 production.

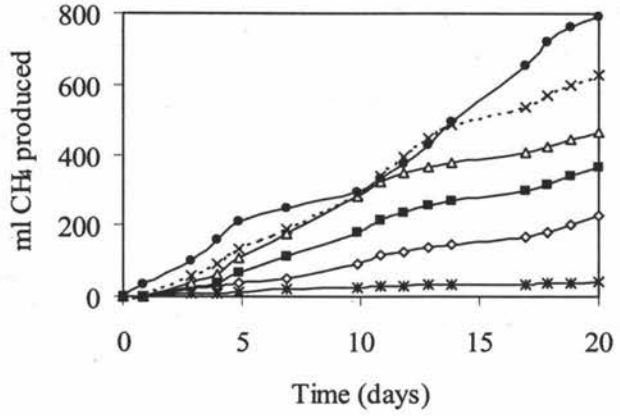


Figure 1. Net cumulative CH_4 production of the reactors fed with VFG-waste and CEP-sludge from $FeCl_3$ pre-treatment (20 days of operation).
Negative control = without addition of buffer
Positive control = with addition of 11.4 g $NaHCO_3$ l^{-1}.

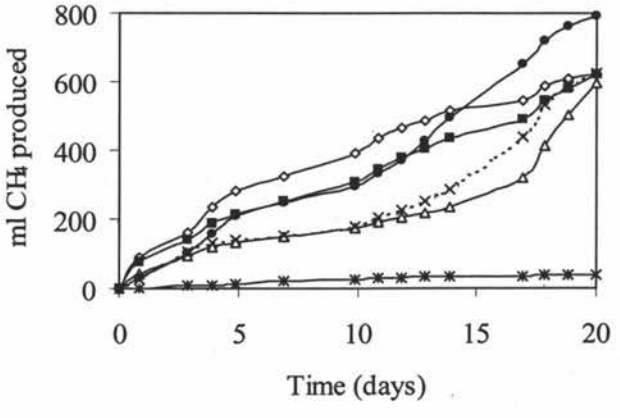


Figure 2. Net cumulative CH_4 production of the reactors fed with VFG-waste and CEP-sludge from WEMOS pre-treatment (20 days of operation).
Negative control = without addition of buffer
Positive control = with addition of 11.4 g $NaHCO_3$ l^{-1}.

Measurement of VS destruction in the reactors showed that the reactor which was strongly inhibited (negative control) had also the lowest VS destruction (Table 2). All the remaining reactors showed VS destruction in the range of 50 to 69%. CH_4 production per g VS destroyed decreased with the increase of CEP-sludge proportion in the reactors. However, comparison of CH_4 production of the CEP-sludge addition reactors and that of the negative control reactor indicated that the addition of CEP-sludge had a positive effect on the digestion process. In terms of CH_4 yield, the substrate could be classified as follows CEP-sludge-$FeCl_3$ < CEP-sludge-WEMOS < VFG-waste.

Examination of the physico-chemical characteristics of the digested residue at the end of the experiment revealed an important drop of pH in the negative control reactor. This decrease corroborated with the accumulation of 5 g VFA - HAc l^{-1}. There was a slight increase of TA observed in the reactor but no BA could be detected. The slight increase of TA was unfortunately not enough to neutralise the acids produced. The VFA / alkalinity ratio of the negative control reactor was thus 30 to 300 times higher than that of the other reactors. In these latter, the buffer capacity had significantly increased and the VFA concentration had significantly decreased. In the CEP-sludge addition reactors, concomitantly to the increase of buffer there was an increase of NH_4^+-N. In the positive control reactor, the increase of NH_4^+-N was rather low compared to the negative control reactor. This indicates that NH_4^+-N was mainly produced from the CEP-sludge.

Except for the negative control reactor, in all the reactors the increase of alkalinity in the effluent fitted the relation of Van Haandel (1994) (Table 2). This relation indicates that, provided the pH of the influent and the effluent are significantly greater than 4.7 (the pKa value of acetic acid) the alkalinity of the effluent can be estimated by the equation:

Alkalinity effluent (meq l^{-1}) = Alkanility influent (meq l^{-1}) + VFA influent (meq l^{-1}) - VFA effluent (meq l^{-1}) + [NH_4^+ effluent (mgN l^{-1}) - NH_4^+ influent (mgN l^{-1})] x 0.071.

Table 2. Results of the performance of the reactors operated for 20 days

Parameters	RF_0	RF_{25}	RF_{50}	RF_{75}	RW_0	RW_{25}	RW_{50}	RW_{75}	RNC	RPC
pH	7.3 (0.1)	7.2 (0.2)	7.2 (0.1)	7.1 (0.1)	7.2 (0.3)	7.3 (0.2)	7.1 (0.1)	7.2 (0.1)	5.4 (0.2)	7.3 (0.2)
BA ($g\ CaCO_3\ l^{-1}$)	1.1 (0.3)	1.1 (0.4)	1.0 (0.2)	0.9 (0.1)	1.7 (0.2)	1.2 (0.3)	1.2 (0.1)	0.9 (0.2)	0	2.3 (0.5)
TA ($gCaCO_3\ l^{-1}$)	4.3 (0.4)	3.8 (0.4)	3.6 (0.2)	2.3 (0.3)	5.3 (0.2)	4.3 (0.1)	3.3 (0.2)	2.6 (0.3)	1.7 (0.1)	6.3 (0.6)
TA theoretical ($gCaCO_3\ l^{-1}$)	4.5	4.0	3.2	1.8	5.6	4.7	4.0	1.9	r.n.a	5.9
VFA-HAc ($g\ l^{-1}$)	0.05 (0.01)	0.17 (0.03)	0.13 (0.02)	0.21 (0.01)	0.09 (0.01)	0.18 (0.04)	0.18 (0.02)	0.22 (0.03)	5.21 (0.01)	0.2 (0.01)
NH_4^+-N ($mg\ l^{-1}$)	211 (12)	199 (10)	180 (11)	138 (6)	595 (8)	398 (6)	269 (9)	155 (8)	81 (6)	100 (5)

Note: RF and RW are related to the reactors containing CEP-sludge from $FeCl_3$ and WEMOS treatment respectively (the subscript indicates the percentage of VFG-sludge); RNC = Negative control; RPC = Positive control; ( ) = Standard deviation; TA theoretic = TA predicted by the relation of Van Haandel (1994); r.n.a = Relation not applicable.

Table 2 (continuation). Results of the performance of the reactors operated for 20 days

Parameters	RF_0	RF_{25}	RF_{50}	RF_{75}	RW_0	RW_{25}	RW_{50}	RW_{75}	RNC	RPC
%VS destroyed	52	54	52	65	58	59	58	61	4	69
VFA / TA	0.01	0.05	0.04	0.09	0.02	0.04	0.06	0.09	3.00	0.03
ml CH_4 g^{-1}VS fed	213	291	292	368	329	338	336	367	22	436
ml CH_4 g^{-1}VS destroyed	411	539	559	569	569	573	580	600	571	629

Note: RF and RW are related to the reactors containing CEP-sludge from $FeCl_3$ and WEMOS treatment respectively (the subscript indicates the percentage of VFG-sludge); RNC = Negative control; RPC = Positive control.

Table 3. Nutrients and heavy metals concentrations in the residues from the reactors operated for 20 days compared respectively with chemical fertiliser and with the limiting values set by the EU and the USEPA

Parameters	RF_0	RF_{25}	RF_{50}	RF_{75}	RW_0	RW_{25}	RW_{50}	RW_{75}	RNC	RPC	CF Doudina *et al.* (1991)	EU Mossakowska *et al.* (1998)	USEPA Chu *et al.* (1998)
N ($g\ kg^{-1}TS$)	44	40	33	38	55	53	53	44	22	35	8 - 11	n.i	n.i
P ($g\ kg^{-1}$ TS)	28	27	14	14	3	7	9	12	8	13	4 - 7	n.i	n.i
K ($g\ kg^{-1}$ TS)	2	5	7	10	1	3	6	7	7	12	6 - 9	n.i	n.i
Ca ($g\ kg^{-1}$ TS)	49	43	38	37	12	18	31	40	31	50	n.i	n.i	n.i
Mg ($g\ kg^{-1}$ TS)	2	3	3	4	4	5	7	7	5	8	n.i	n.i	n.i
Fe ($g\ kg^{-1}$ TS)	202	138	86	42	38	39	27	15	6	10	n.i	n.i	n.i
Zn ($mg\ kg^{-1}$ TS)	1221	857	757	488	1061	897	767	435	275	446	n.i	2500 – 4000	101- 49000

Note: n.i = Not indicated; RF and RM are related to the reactors containing CEP-sludge from $FeCl_3$ and WEMOS treatment respectively (the subscript indicates the percentage of VFG-sludge); RNC = Negative control; RPC = Positive control; CF = Chemical fertiliser; EU = European Union; USEPA = United State Environment Protection Agency.

Table 3 (continuation). Nutrients and heavy metals concentrations in the residues from the reactors operated for 20 days compared respectively with chemical fertiliser and with the limiting values set by the EU and the USEPA

Parameters	RF_0	RF_{25}	RF_{50}	RF_{75}	RW_0	RW_{25}	RW_{50}	RW_{75}	RNC	RPC	CF Doudina *et al.* (1991)	EU Mossakowska *et al.* (1998)	USEPA Chu *et al.* (1998)
Cu (mg kg^{-1} TS)	205	146	101	72	167	155	93	76	42	67	n.i	1000 – 1750	84 - 17000
Cd (mg kg^{-1} TS)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.i	20 – 40	1 - 3410
Ni (mg kg^{-1} TS)	93	71	42	38	68	51	28	21	9	14	n.i	300 – 400	2 - 5300
Cr (mg kg^{-1} TS)	24	15	15	16	19	16	17	15	9	14	n.i	n.i	10 - 99000
Pb (mg kg^{-1} TS)	102	83	49	35	68	55	28	17	n.d	n.d	n.i	750 – 1200	13 – 26000
Hg (mg kg^{-1} TS)	11	6	3	1	4	2	1	1	n.d	n.d	n.i	16 - 25	n.i

Note: n.d = Not detected; n.i = Not indicated; RF and RM are related to the reactors containing CEP-sludge from $FeCl_3$ and WEMOS treatment respectively (the subscript indicates the percentage of VFG-sludge); RNC = Negative control; RPC = Positive control; CF = Chemical fertiliser; EU = European Union; USEPA = United State Environment Protection Agency.

It is also important to note that the residues from the reactors (Table 3) contained more plant nutrients than their raw counterparts (Table 1).

Effect of the digestion time on the performance of the reactors

Overall destruction efficiencies of the reactors gradually increased with increase of digestion time (Figures 3 and 4). As a result of an increasing VS destruction, CH_4 production per g VS fed concomitantly increased.

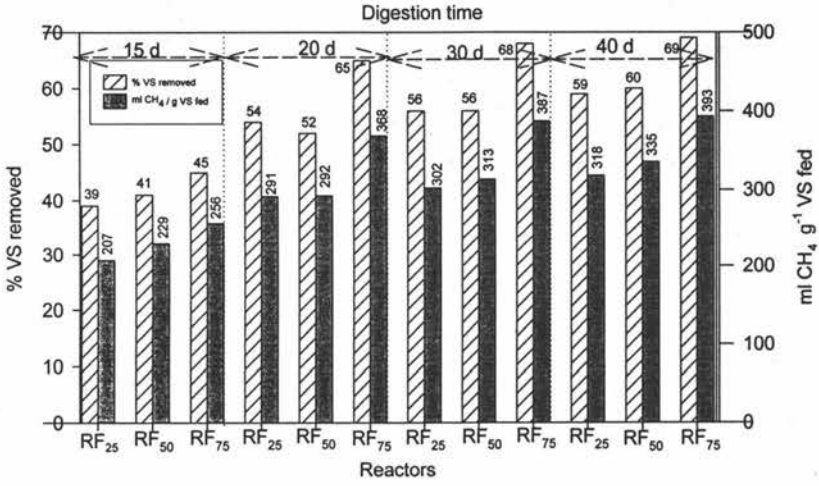


Figure 3. Effect of digestion time on the performance of the reactors fed with VFG-waste and CEP-sludge from pre-treatment with $FeCl_3$.

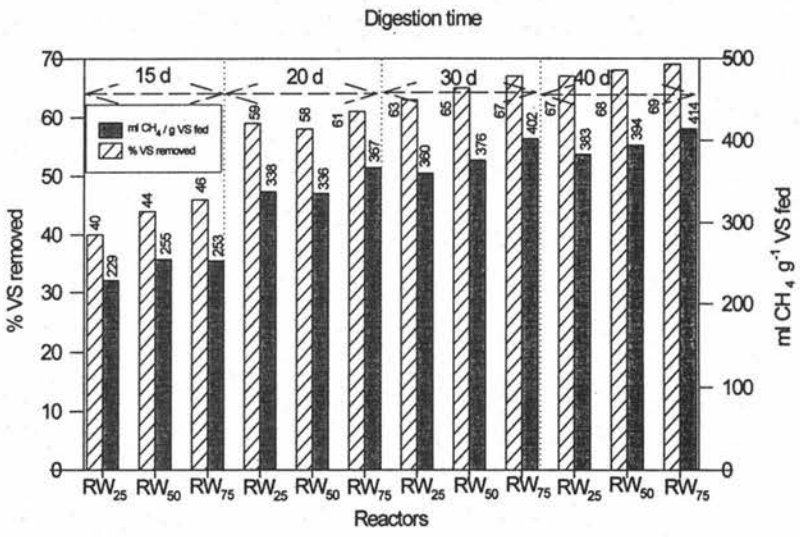


Figure 4. Effect of digestion time on the performance of the reactors fed with VFG-waste and CEP-sludge from pre-treatment with WEMOS.

DISCUSSION

Addition of CEP-sludge

Alkalinity is a measure of the capacity of water or sludge to neutralise a strong acid. Tchobanogous & Burton (1991) report that alkalinity of primary sewage sludge range between 500 to 1500 mg $CaCO_3$ l^{-1}. They however did not indicate whatever it is BA or TA. Their values are similar to the BA measured in CEP-sludge from $FeCl_3$ and CEP-sludge from WEMOS treatment. Yet, they are two to eight times lower than the TA observed. It should be reminded that besides HCO_3^- and CO_3^{2-}, TA is also attributable to other components such as phosphate and hydroxide. The latter are to a large

extend precipitated during the chemical pre-treatment and may therefore contribute to increase the alkalinity of the wasted sludge.

Anaerobic digestion

The combined anaerobic digestion of CEP-sludge and VFG-sludge was investigated in batch reactors for 20 days. The latter time period corresponds to the sludge retention time generally applied in solid waste reactors (St-Joly, 1992; Szikrist *et al.*, 1988; De Baere & Verstraete, 1984). During the anaerobic digestion process, the negative control was strongly inhibited. The low CH_4 production and the low pH of the reactor is in accordance with the fact that methanogens have their optimal pH around 7.0 to 7.5 while acidogens can still survive at pH < 4 (Champiat, 1994). The large proportion of CO_2 in the biogas confirmed that acidogenesis still occurred. This resulted in the accumulation of VFA and the consumption of the bicarbonate alkalinity (HCO_3^-) as described by the well-known reaction R-COOH + HCO_3^- → $RCOO^-$ + H_2O + CO_2. The latter reaction evidences that during generation of VFA, HCO_3^- is consumed and CO_2 is produced.

All the other reactors, except the negative control, had a good VS destruction and good CH_4 generation. This observation correlates with the low VFA/Alkalinity ratio of the effluent, which indicates that the reactors were stable. A VFA / Alkalinity ratio above 0.3 indicates an instability of the reactor (Ross *et al.*, 1992).

The better stability of the CEP-sludge addition reactors was due the presence of sufficient buffer supplied by the combination of CEP-sludge with VFG-waste. Indeed the buffer/substrate solid ratio of these reactors was comprised between 0.025 and 0.2, which is the range necessary for efficient treatment (Buivid *et al.*, 1981). Thus, the buffer allowed increasing the resistance of the reactors to acidification. Since the reactors operated well, the effluent alkalinity fitted the relation of Van Haandel (1994). It is therefore concluded that alkalinity increased in the reactors basically through VFA conversion to

CH_4 ($CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$) and ammonification ($RNH_2 + 2H_2O \rightarrow ROH + NH_4^+ + OH^-$).

Combining CEP-sludge and VFG-waste resulted in decreasing the C/N ratio of the mixtures. A C/N ratio of 30 is often cited as optimum for anaerobic digestion (Borda, 1980). However in view of the present results, it can be concluded that the decrease in C/N ratio has no negative effect on the digestion process. This observation substantiates the report of Gunnerson & Stuckey (1986) which indicate that C/N ratio from less than 10 to over 90 can still lead to efficient digestion.

Apart from the effect of CEPS, the experimental results imply that the digestion time plays an important role in the anaerobic digestion of solid waste. The results suggest that longer digestion times are preferable above the short ones.

The increase of nutrient level in the residue (Table 3) is due to a concentration effect since part of the dry matter was degraded. The rich nutrient content of the residues, compared to the nutrient level of several chemical fertilisers (Table 3), make them applicable for practical use on farmland. This is indeed conceivable since the level of heavy metals in the residues was lower than the limits fixed by European Union (EU) and the United State Environment Protection Agency (USEPA) as well (Table 3).

Cost saving from CEP-sludge co-digestion with VFG-waste

The major advantage of combining anaerobic treatment of CEP-sludge and VFG-waste is related to the economic benefits. To guarantee an efficient treatment, a buffer / substrate solid ratio of 0.025 to 0.2 is needed (Buivid *et al.*, 1981). Since 1 kg of $NaHCO_3$ is equivalent to about 0.6 g $CaCO_3$, this implies that one needs 42 to 330 kg $NaHCO_3$ per ton of TS.

1 ton of $NaHCO_3$ cost about 375 EURO. Thus the treatment cost for 1 ton of TS can implicate 16 to 124 EURO in terms of buffering agent. By combining CEP-sludge and VFG-waste one can therefore save substantially.

Optimal CEP-sludge:VFG-waste ratio

The results showed that the higher the CEP-sludge proportion, the lower was the CH_4 generated per gram VS destroyed. This is related to the fact that CEP-sludge has a low energy content, as indicated by their low COD/VS ratio, compared to VFG-waste (Table 1). Moreover, it should also be noted that increasing the CEP-sludge proportion correlated with increasing the heavy metals content in the mixture. From the overall results obtained in this study, the proportion 25% CEP-sludge and 75% VFG-waste appears to be technically the best advice.

In terms of heavy metals content, CEP-sludge from WEMOS pre-precipitation will be less susceptible to regulatory restrictions of the disposal on agricultural lands. However in both situations, the application of the digested residue on farmland should be evaluated case by case, taking in consideration the concentration of metals in the soil and also the pH of the soil.

CONCLUSIONS

A combined anaerobic digestion of CEP-sludge from domestic wastewater and VFG-waste was investigated in lab-scale batch reactors. From this study, the following conclusions can be drawn:

1. At 50 g TS l^{-1} reactor, VFG-waste could not be efficiently degraded without the supply of sufficient buffer capacity.
2. CEP-sludge has a high buffer capacity and can therefore be used, instead of chemical buffer such as $NaHCO_3$, to prevent the acidification of the reactors.
3. During the breakdown of the CEP-sludge, there is an increase of the buffer capacity of the reactor due to VFA conversion and ammonification.

4. The higher the proportion of CEP-sludge, the lower the amount of CH_4 generated per gram of VS destroyed because of the low energy content of the former and the higher the amount of heavy metals in the final digested residues.
5. The ratio 25% CEP-sludge and 75 %VFG-waste appears to be technically the best practical combination.
6. During the combined digestion of CEP-sludge with VFG-waste the efficiency of the reactor increases with the increase of digestion time.

ACKNOWLEDGEMENTS

This research was partially supported by a grant from the Government of Côte d'Ivoire. The authors thank Ing. Johan Vermeulen and Organic Waste Systems (OWS) for providing the VFG-waste and the anaerobic digested VFG inoculum.

CHAPTER VIII

CONCLUSIONS AND PERSPECTIVES

Kalogo Y. & Verstraete W.

Part of this text was published as:

Hammes F. Kalogo Y. & Verstraete W. (2000) Anaerobic digestion technologies for closing the domestic water, carbon and nutrient cycles. *Water Science & Technology* **41**(3), 203-211

CONCLUSIONS AND PERSPECTIVES

There is an interest to use UASB reactors for the anaerobic treatment of domestic wastewater. The main reasons for this are the low investment, operation and sludge disposal costs and low space requirement for the UASB reactor (Van Haandel & Catunda, 1997). The investment costs of a UASB reactor treating domestic wastewater may further decrease since it can be started without seed sludge, based on the autochthonous microbial communities of the wastewater. Indeed, both anaerobic and facultative anaerobic microorganisms are present in domestic wastewater (Crowther & Harkness, 1975; Bitton, 1999). Overall literature reports on self-inoculation suggest that the process may need to be optimised in view of the slow start-up. One of the major points of concern for domestic wastewater is that about 45 to 55% of the CODt are in the form of SS (Tchobanoglous & Burton, 1991). During reactor operation, poorly biodegradable SS tend to accumulate in the reactor. Mathematical modelling has shown that this results in a gradual decrease of the activity of the anaerobic biomass (Rozzi & Verstraete, 1981). Another major point of concern for domestic wastewater treatment in the UASB reactor is the poor granular sludge formation. With the successful experiences of granular sludge formation on agroindustrial wastewater, the situation of domestic wastewater is currently ascribed to the low content of carbohydrates in the latter (Verstraete & Vandevivere, 1999). Indeed, domestic wastewaters generally contain only 10 to 40 mg carbohydrates l^{-1} on a total of 210 to 740 mg COD l^{-1} (Henze *et al.*, 2000). It is quite conceivable that one could import granular sludge from full-scale UASB reactors treating agroindustrial wastewater where granules are readily produced. Yet granular sludge has a market price of about 1.5 to 2.5 Euro per kg VSS. This granular biocatalyst will, when used to treat domestic wastewater, perform but not grow because the SS tend to form a barrier around them (Sayed & Fergala, 1995). As a consequence, under long-term feeding conditions the granules may disintegrate. One may also use a reactor with flocculent sludge bed. Yet such a sludge bed can be easily washed out when a high hydraulic load is applied and this can cause system failure.

This research work particularly focused on the above mentioned aspects of the anaerobic treatment of domestic wastewater in the UASB reactor.

A thorough investigation of self-inoculation confirmed that the process is quite feasible (Chapter II). Moreover, this investigation provided a better understanding of the process dynamics. Earlier investigators reported biogas recovery between 80 to 115 ml g^{-1}COD added (Grin *et al.*, 1983; Barbosa & Sant'Anna, 1989) or 50 to 100 ml g^{-1} COD removed (Draaijer *et al.*, 1991). During the present study, the reactor removed up to 80% CODt and 90% SS; yet the biogas recovery was only 70 ml g^{-1} COD added or 107 ml g^{-1} COD removed. This low biogas recovery is due to the fact that interception of organic matter is the most dominant process during the start-up of a self-inoculated reactor. The results indicated that induction of biochemical reactions starts slowly and therefore causes a discrepancy between COD removed and biogas recovered. This opened the question of how to shorten the time needed to induce an efficient anaerobic biocatalyst.

Recent reports have indicated that natural polymers, e.g. chitosan, are capable to enhance the start-up of anaerobic reactors (El-Mamouni *et al.*, 1998). Therefore, effort has been focused on the examination of the effect of polymer dosage to the feed. The water extract of *Moringa oleifera* seeds (WEMOS) was used in this respect (Chapter III). WEMOS is not sensitive to a pH variation in the range of 4.0 to 9.5 because it has an isolectric point between 10 and 11 (N'Dabigengesere *et al.*, 1995). It is capable of removing SS from wastewater and it contributes as such to the removal of total coliforms. It also contains several nutrients (e.g. glucose, N, P, metal ions and free amino acids) capable to support microbial growth. The dosage of 2 ml WEMOS (2.5%) l^{-1} of wastewater (without primary sedimentation) before passing through the reactor allowed to shorten the biological start-up (biogas recovery) of the reactor by 20%. The addition of WEMOS does not affect the gas production so intensely compared to the control reactor, that it may not be economic to continue to dose it after gas production has started. The dose of WEMOS did not lead to the formation of granules, yet it showed a positive effect on the aggregation of bacteria. Moreover the WEMOS addition reactor had a wider hydrolytic microbial diversity (Chapter IV). This may explain the rapid start-up of that reactor compared to the control. The results underlined a new application of WEMOS and opens perspectives for upgrading UASB reactors.

Granular sludge was successfully developed on domestic wastewater, with flocculent sewage sludge, simply by elevating the upflow velocity (V_{up}) of the reactor from 1 m h^{-1} to 5 m h^{-1} (through the recycle flow) and inoculating the reactor with the proper amount of sludge (Chapter V). Tracer experiments with NaCl indicated that the hydraulic mixing intensity in the reactor is moderate (d = 0.088) at V_{up} = 1 m h^{-1} and high (d = 0.309) at V_{up} = 5 m h^{-1}. It appears that to promote granule formation a mixed flow reactor is to be preferred over a plug-flow reactor. This agrees with the results reported by Noyala & Morena (1994) which indicated that flocculent anaerobic sludge can be converted to a relatively active granular sludge, only using hydraulic stress. The results clearly showed that carbohydrates (molasses) were not the key factor in promoting granule formation. Yet molasses have the main advantage in increasing the biomass activity and the biogas production. This opens the following question: which factor is responsible for successful granulation on concentrated agro-industrial wastewater? We postulate that this may result from the high loading applied in those reactors and the subsequent high rate of biogas production. Indeed, UASB reactors treating agro-industrial wastewater operated at a volumetric loading rate (B_v) of 14-27 kg COD m^{-3} d^{-1} and produced 5-6 CH_4 m^3 m^{-3} d^{-1} (Pette & Versprille, 1981; Grusenmeyer & Pipyn, 1987; Souza *et al.*, 1992). The biogas contributes to increase the mixing intensity into the reactor and therefore create the necessary hydraulic stress conditions for granulation. With domestic wastewater the B_v is rarely higher than 6 kg m^{-3} d^{-1} and biogas production is low, e.g. 0.2-0.5 m^3 CH_4 m^{-3} d^{-1} (Monroy *et al.*, 2000; Kalogo & Verstraete, 1999). This low amount of biogas practically does not help to increase the turbulence in the reactor. Regarding the full-scale UASB plants that are currently operating for the treatment of domestic wastewater, it may be technically difficult to increase the upflow velocity through recycling of part of the effluent. In this respect, co-supply of carbohydrates may be advised in order to increase biogas production, provided it is available at low cost.

Facing the negative impact of SS on UASB reactor, there is a consensus that at least part of the SS should be removed prior to feeding the reactor (Rebac, 1998; Vander Last & Lettinga, 1992; De Man *et al.*, 1988). Effort was further focused on increasing the CODs/VSS ratio to about 10 prior to anaerobic treatment. A novel system, CEPS-UASB, was therefore investigated (Chapter VI). This system

combines a chemically enhanced primary sedimentation (CEPS) and subsequent anaerobic treatment. A satisfactory increase of CODs/VSS was achieved by pre-treating raw domestic wastewater with $FeCl_3$ and WEMOS at a short HRT, e.g. 1 h. The total system achieved on average 80% CODt removal and 90% SS removal at HRT of 3 hours. During the treatment of the supernatant fluid, no decrease in activity of the granular sludge was observed. This supports the idea that one should increase the CODs/VSS to about 10 to keep the anaerobic sludge sufficiently active (De Baere & Verstraete, 1982). The system is promising since it should decrease the volume needed by a classical one-step UASB reactor by a factor 0.4. This suggests that for future UASB reactors dedicated to the treatment of domestic wastewater, CEPS should be implemented. The process may also be easily introduced in, already existing, full-scale plants. For those cases, this means that a large water flow could be daily treated. The WEMOS increased the nutrient concentration in the final effluent. The use of WEMOS should thus be interesting in case the effluent of the UASB reactor has to be reused on farmland deficient in nitrogen and phosphorus. Yet, a strong disinfection step will be necessary to meet the international standards (TC and FC < 3 Log cfu 100 ml^{-1}) of water reuse in agriculture (WHO, 1989). $FeCl_3$ should be advised in case the UASB effluent will be discharged in the river.

Coupling CEPS to UASB reactor poses inevitably the question of management of the chemically enriched primary (CEP)-sludge wasted. Anaerobic treatment of CEP-sludge was shown to be feasible yet with low biogas yield compared to naturally sedimented sludge (Gossett *et al.*, 1978; Ghyoot & Verstraete, 1997). Ghyoot & Verstraete (1997), however, observed an increase of alkalinity during the treatment of CEP-sludge due to an increase of ammonium nitrogen. The feasibility of co-digestion of CEP-sludge together with vegetable fruit and garden (VFG)-waste was investigated (Chapter VII). This option appears quite feasible and promising in terms of saving on chemical buffer, e.g. $NaHCO_3$. Increased alkalinity due to the breakdown of CEP-sludge results in the stabilisation of the system. The combined treatment also offers the possibility to recover a much higher amount of biogas that can be upgraded for instance as electricity. Yet attention should be given to the concentration of heavy metals that can be introduced by the CEP-sludge in case the anaerobic digested residue has to be used on farmland.

Based on the overall results described above, a novel integrated concept for future anaerobic treatment of domestic wastewater including solid waste digestion is proposed. This concept is represented by Figure 1. The proposed system includes chemical enhanced sedimentation to remove SS in the form of concentrated sludge. The chemical must be dosed to improve the SS removal so that the CODs/VSS ratio of the supernatant fluid can be increased to about 10 to facilitate suitable treatment. This supernatant is subsequently digested in a UASB reactor. The sludge from the chemical sedimentation is co-digested with VFG-waste in a completely stirred tank reactor (CSTR) or a dry anaerobic reactor (De Baere & Verstraete, 1985) with the main objective of producing energy. Finally, the energy produced is converted to electricity to operate an ozonator or an UV light for the disinfection of the UASB reactor effluent. This effluent can then be used for agriculture. Ozone production does not necessarily require pure oxygen since it can also be generated from air (Tchobanoglous & Burton, 1991) so it can be produced everywhere, provided electricity is available. Besides its disinfection effect, ozone has also the advantage of reducing odour associated with anaerobic effluent (Takaski 1998). Odour problems have been reported to be a determining factor for the acceptance of UASB technology by people in Colombia (Kalker *et al.*, 1999). The major originality of this process is that biogas from the treatment of domestic wastewater is now recovered in an environmentally friendly way and upgraded. Compared to the two-stage reactor (Wang, 1994), this novel process implies only two reactors. A pilot-scale investigation is necessary to study in details the technical feasibility of the process.

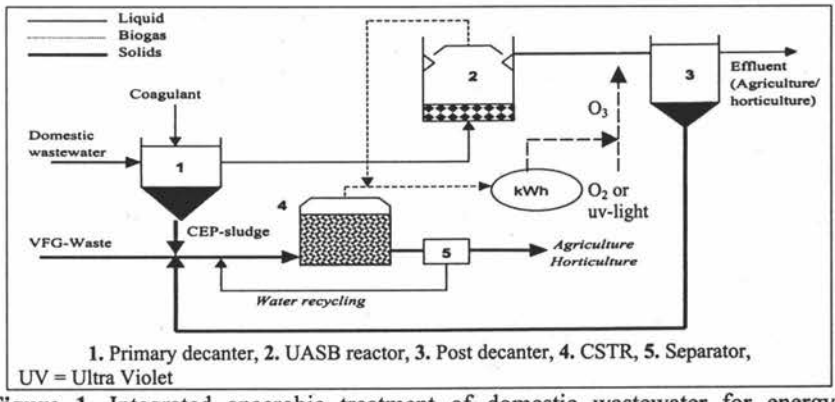


Figure 1. Integrated anaerobic treatment of domestic wastewater for energy recovery and regeneration of water and bio-solids (carbon and nutrients)

The concept could be design according to 3 steps as follows:

(1) Start-up of the UASB reactor by self-inoculation using raw domestic wastewater. During this step, WEMOS or an alternative coagulant at least partially biodegradable has to be continuously added to the wastewater till biogas production is observed. The V_{up} in the reactor has to be kept to around 1 m h^{-1} to avoid the loss of biomass. Loss of biomass during this step could indeed slow down the start-up.

(2) When sufficient biomass is retained inside the reactor the V_{up} can be increased in increasing the recirculation flow of part of the effluent. The goal of this second step is to provide a good mixing of the sludge and therefore favour granule formation.

(3) After granulation of the anaerobic biomass, the reactor has to be retrofitted by a CEPS treatment using WEMOS, if it is widely available, as coagulant. Otherwise, synthetic coagulants such as $FeCl_3$ can be used. At this step a solid waste reactor should be integrated to the concept. The solid waste reactor will serve to treat the chemically enriched primary sludge from the CEPS together with organic solid waste. The reactor will therefore operate without the need to supply buffer like $NaHCO_3$. It will also allow the recovery of an important amount of biogas. This biogas can be combined with the biogas from the UASB reactor. Finally, the total biogas produced can be converted to electricity to operate an ozonator or an UV light for the disinfection of the UASB reactor effluent. This effluent can then be used for agriculture.

SUMMARY

Literature study (Chapter I) has shown that the upflow anaerobic sludge blanket (UASB) reactor developed at the beginning of the eighties for the treatment of agroindustrial wastewater is being tentatively used for the treatment of domestic wastewater. Although encouraging results were obtained, some operating problems due to the characteristics of domestic wastewater were observed. Three important problems currently relate to the operation of the reactor, e.g. 1) slow start-up of the reactor when no seed sludge is used, 2) poor or no granular sludge formation, 3) accumulation of poorly biodegradable SS in the reactor, and thereby leading to a decrease in biomass activity and COD to CH_4 conversion efficiency. The message from that literature study also indicated some "potential" solutions, which needs to be investigated.

The main objective of this work was to find some solutions to the problems mentioned above and to establish the basis of the feasibility of an integrated concept for the anaerobic treatment of domestic wastewater.

Preliminary investigations (Chapter II) had a primary goal of gaining more scientific knowledge that underpins the process of self-inoculation. The dynamics of self-inoculation were examined in lab-scale UASB reactor, 2.3 l working volume, for 154 days at HRT of 4 h and B_v of 1.99 ± 0.22 g COD l^{-1} d^{-1}. Statistical analysis of the results by LSD and CUSUM indicated two types of start-up periods. During the first period (day 1 - day 40) the reactor achieved CODt and SS removal efficiencies up to 65% and 73% respectively, mainly by interception of organic matter. The second period (from day 104) was expressed by effective biogas release. The interval between those two periods was characterised as an induction time of biochemical reactions. The methanogenic activity increased in time as follows, 0.01 (day 42), 0.02 (day 84), 0.06 (day 126) and 0.1 g COD-CH_4 g^{-1}VSS d^{-1} (day 154). The biogas production over the experimental period was low, 107 ml g^{-1} COD removed, although the reactor removed up to 80% CODt and 90% SS. The discrepancy between the biogas produced and the COD removed was probably due to the long time it took before

the second start-up became efficient. Based on the overall observation, the dynamics of the process was represented by a simple descriptive model including the pH evolution of the effluent, VFA concentration in the effluent and biogas production.

The effect of dosage of a natural polymer, a water extract of *Moringa oleifera* seeds (WEMOS), to the wastewater (without primary sedimentation) before passing through a non-inoculated UASB reactor was therefore investigated for 154 days and compared with a control reactor (Chapter III). Preliminary batch experiments in jar tests confirmed that WEMOS is effective in SS removal and contributes as such to reduce the total faecal coliform concentration in wastewater. The performance of WEMOS was also not sensitive to a pH variation in the range of 4.0 to 9.5.

The continuous dosage of 2 ml WEMOS (2.5%) l^{-1} improved slightly but significantly (as shown by t-test on pared observation) the physical performance of the reactor. The addition of WEMOS shortened the second start-up period of the reactor by 20%. This correlated with an increase of the acidogenic and methanogenic activities by a factor 2.4 and 2.2 respectively compared to that of the control reactor. Physico-chemical analysis of the biomass from the reactors revealed that the WEMOS addition reactor was enriched in metal ions supporting the growth of methanogens. No granular sludge was formed during that experiment. Yet scanning electron microscopic examination (SEM) of the flocculent biomass revealed a significant positive effect of WEMOS on the aggregation of bacteria.

Microbiological analysis (Chapter IV) showed that the biomass from the WEMOS addition reactor had a wider hydrolytic microbial diversity with *Enterobacter* and *Klebsiella* as dominant bacteria. This was explained by the complex composition of WEMOS. Further investigations with 17 strains of pure cultures of hydrolytic bacteria (Gram positive and Gram negative) demonstrated that those bacteria could indeed grow on media consisting of WEMOS as sole source of carbon and nutrient (e.g. N, P, metal ions and free amino acids). A close correlation between the microbial diversity and the reactor start-up was observed

by means of batch reactor studies. It was observed that a reactor with a wider diversity of hydrolytic bacteria could profit from a rapid biological start-up.

Granular sludge formation in the UASB reactor inoculated with flocculent biomass was investigated using hydrodynamic conditions in the reactor and co-supply of an energy rich carbohydrate, e.g. molasses, to the wastewater for 211 days (Chapter V). Sludge bed expansion and tracer experiments have shown that the mixing intensity in the reactor is related both to the V_{up} and the amount of biomass in the reactor.

Continuous experiments indicated that molasses was not the key factor in promoting granules although 21% of the COD supplied to the reactor consisted of molasses. Yet it had the advantage of improving the activity of the biomass by increasing the development of acid formers. Increase of V_{up} from 1 m h^{-1} to 5 m h^{-1} improved the mixing intensity in the reactor through a homogeneous distribution of water. As a result, granular sludge, with 1 mm diameter on average, was formed on domestic wastewater after about two months of operation. SEM of the granules showed coccoid bacteria on the surface layer. The inner part indicated mainly rod-shaped bacteria resembling *Metanosaeta*. At V_{up} of 5 m h^{-1} the efficiency of the interception of SS into the reactor decreased. The SS removal efficiency was reduced from 65% (V_{up} of 1 m h^{-1}) to 52% (V_{up} of 5 m h^{-1}) due to wash out.

A novel system, CEPS-UASB, for the treatment of domestic wastewater was investigated for a period of 170 days (Chapter VI). This system combines a chemically enhanced primary sedimentation (CEPS) and subsequent anaerobic treatment. The CEPS with 70 mg $FeCl_3$ l^{-1} (day 1 - day 82) and 24 ml WEMOS (5%) l^{-1} (day 83 - day 170) improved the CODs/VSS ratio of the raw wastewater from 1.4 to 5 and 15 respectively. The system achieved up to 80% CODt removal and 90% SS removal at HRT of 3 hours. The CEPS-UASB system may reduce the total volume needed by a classical UASB reactor by a factor 0.4 to achieve the same efficiency.

The anaerobic co-digestion of the chemically enriched primary (CEP)-sludge together with vegetable fruit and garden (VFG)-waste was investigated in batch reactors for different CEP-sludge/ VFG-waste ratio (Chapter VII). The results showed that the co-digestion of both substrates is compatible. 50 to 69% VS destruction was obtained at a load of 50 g TS l^{-1} reactor and a retention time of 20 days. Furthermore the CEP-sludge made the anaerobic breakdown of VFG-waste possible without addition of extra alkalinity, e.g. $NaHCO_3$, for pH stabilisation. This was due to the high buffering capacity of the CEP-sludge and the increase of alkalinity during the operation of the reactors basically through VFA conversion to CH_4 and ammonification of nitrogen from CEP-sludge. For further applications, the use of a minimal amount of CEP-sludge (25%) will be necessary to avoid excessive concentration of heavy metals in the digested residue.

In conclusion, an integrated concept for the anaerobic treatment of domestic wastewater including solid waste digestion is proposed (Chapter VIII). The potential advantages of this system are (1) recovery of energy from organic waste in an environmentally friendly way, (2) lowering the negative effect of SS in the UASB reactor, (3) production of a high quality effluent for irrigation. Those advantages should be confirmed by the design of a pilot plant.

SAMENVATTING

Literatuuronderzoek (Hoofdstuk I) toonde aan dat er pogingen werden ondernomen om de opstroom anaërobe slib bed reactor (UASB) – ontworpen begin jaren tachtig voor de behandeling van agro-industrieel water – te gebruiken voor de behandeling van huishoudelijk afvalwater. Niettegenstaande de bemoedigende resultaten kwamen enkele problemen in de bedrijfsvoering, te wijten aan de karakteristieken van het afvalwater, aan de oppervlakte. Drie voorname problemen zijn tegenwoordig gekoppeld aan de werking van de reactor, 1) trage opstart van de reactor indien geen inoculatieslib gebruikt wordt, 2) slechte vorming of geen vorming van granulair slib, 3) opstapeling van laag biodegradeerbare zwevende stoffen (ZS) in de reactor, wat aanleiding geeft tot een vermindering in biologische activiteit en een lagere efficiëntie in de CZV-verwijdering. De literatuurstudie schuift ook enkele "mogelijke" oplossingen naar voren voor, die verder dienen onderzocht te worden.

De hoofddoelstelling van deze doctoraatsthesis is oplossingen te vinden voor bovengenoemde problemen en een basis te leggen voor de bruikbaarheid van een geïntegreerd concept voor de anaërobe behandeling van huishoudelijk afvalwater.

Het voornaamste objectief van de preliminaire studies (Hoofdstuk II) was het vergaren van meer wetenschappelijke kennis om het proces van zelf-inoculatie te onderbouwen. De dynamiek van zelf-inoculatie werd onderzocht in een UASB-reactor op laboschaal met een werkvolume van 2.3 l, gedurende 154 dagen aan een hydraulische verblijftijd (HVT) van 4 uur en een volumetrische belasting (B_v) van 1.99 $\pm$ 0.22 g COD l^{-1} d^{-1}. Statistische analyse van de resultaten door LSD en CUSUM, toonden twee types van opstartperiodes aan. In de eerste periode (dag 1 – dag 40) bereikte de reactor CZV- en ZS-verwijderingsefficiënties tot respectievelijk 65 % en 73 %, vooral door het weerhouden van organisch materiaal. De tweede periode (vanaf dag 104) kwam tot uiting door het effectief vrijkomen van biogas. Het interval tussen deze twee periodes werd gakarakteriseerd als een inductietijd van de biochemische reacties. De methanogene activiteit vermeerderde in de tijd als volgt, 0.01 (dag 42), 0.02 (dag 84), 0.06 (dag 126) en 0.1 g COD-CH_4 g^{-1}VSS d^{-1} (dag 154). De biogasproductie over de experimentele periode was met 107 ml g^{-1} CZV-verwijdering laag, hoewel de reactor tot 80 % CZV en 90 % ZS verwijderde. De

tegenstrijdigheid tussen de hoeveelheid geproduceerd biogas en de hoeveelheid CZV-verwijdering was waarschijnlijk te wijten aan de lange tijd die nodig was vooraleer de tweede opstart efficiënt werd. Algemeen kon vastgesteld worden dat de dynamiek van het proces te beschrijven was door een eenvoudig model waartoe de pH-evolutie van het effluent, de VVZ- concentratie in het effluent en de biogasproductie behoorden.

Het effect van de toediening van een natuurlijk polymeer - een waterextract van *Moringa oleifera* zaden (WEMOS) - aan het afvalwater (zonder primaire sedimentatie) vooraleer door een niet-geïnoculeerde UASB-reactor te gaan, werd onderzocht gedurende 154 dagen en vergeleken met een controlereactor (Hoofdstuk III). Preliminaire experimenten bevestigden dat WEMOS effectief werkt in de ZS-verwijdering en dat het op zich bijdraagt om de totale concentratie aan fecale coliformen in het afvalwater te verminderen. Daarbij was de werking van WEMOS niet afhankelijk van pH- variaties tussen van 4.0 tot 9.5.

De continue dosering van 2 ml WEMOS (2.5 %) l^{-1} verbeterde de fysische werking van de reactor licht, doch significant (aangetoond met een t-test op gepaarde waarnemingen). De toevoeging van WEMOS verkortte de tweede opstarttijd van de reactor met 20 %. Dit was gecorreleerd met een toename van de acidogene en methanogene activiteit met respectievelijk een factor 2.4 en 2.2, ten opzichte van de controlereactor. Fysicochemische analyse van de biomassa in de reactoren toonde aan dat de reactor met WEMOS een aanrijking vertoonde in metaalionen wat de groei van methanogenen bevorderde. Tijdens dit experiment werd er geen granulair slib gevormd. Toch toonde onderzoek van de geflocculeerde biomassa met een scanning elektronenmicroscoop (SEM) aan dat WEMOS een significant positief effect had op de aggregatie van bacteriën.

Na microbiologische analyse (Hoofdstuk IV) bleek dat de biomassa uit de WEMOS-reactor een bredere hydrolytische microbiële diversiteit bezat met *Enterobacter* en *Klebsiella* als dominerende soort. Dit werd verklaard door de complexe samenstelling van WEMOS. Verder onderzoek met 17 stammen uit pure culturen van hydrolytische bacteriën toonde aan dat deze bacteriën konden groeien op media met WEMOS als enige bron van koolstof en nutriënten (bv. N, P, vrije aminozuren en metaalionen). Een nauw verband tussen de microbiële diversiteit en de opstart van de reactor werd

waargenomen via batchreactor onderzoek. Er werd waargenomen dat een reactor met een bredere diversiteit aan hydrolytische bacteriën voordeel kon halen uit een snelle biologische opstart.

De vorming van granulair slib in de UASB- reactor die geïnoculeerd was met geflocculeerde biomassa, werd tijdens 211 dagen onderzocht door middel van hydrodynamische condities in de reactor en toevoeging van een energierijke koolwaterstof, bv. melasse, aan het afvalwater (Hoofdstuk V). Uitzetting van het slibbed en tracer experimenten bewezen dat de mengintensiteit in de reactor in relatie staat tot V_{up} en de biomassa in de reactor.

Uit continue experimenten bleek dat de melasses niet de hoofdfactor waren in het bevorderen van granulevorming, niettegenstaande 21 % van de CZV, toegediend in de reactor, uit melasse bestond. Toch hadden ze het voordeel dat de biologische activiteit verbeterde door de ontwikkeling van meer zuurvormende bacteriën. Een stijging van V_{up} van 1 m h^{-1} tot 5 m h^{-1} verbeterde de mengintensiteit in de reactor door een homogene verdeling van water. Na een periode van ongeveer twee maanden werden slibgranules van gemiddeld 1 mm diameter bekomen. Onderzoek van de granules met SEM liet coccoïde bacteriën aan de oppervlaktelaag zien. In het binnenste deel kwamen voornamelijk staafjesvormige bacteriën, gelijkend op *Metanosaeta,* voor. Bij een V_{up} van 5 m h^{-1}, was de efficiëntie bij het weerhouden van SS in de reactor gedaald. De verwijderingsefficiëntie verminderde door wash-out van 65 % (V_{up} van 1 m h^{-1}) tot 52 % (V_{up} van 5 m h^{-1}).

Een nieuw systeem, CEPS-UASB, voor de behandeling van huishoudelijk afvalwater werd onderzocht gedurende een periode van 70 dagen (Hoofdstuk VI). Dit systeem combineert een chemisch bevorderde primaire sedimentatie (CEPS) met een daaropvolgende anaërobe behandeling. De CEPS met 70 mg $FeCl_3$ l^{-1} (dag 1 – dag 82) en 24 ml WEMOS (5 %) l^{-1} (dag 83 – dag 170) verbeterde de CZVs/VSS verhouding van het ruwe afvalwater van 1.4 tot 5 en 15 respectievelijk. Dit systeem behaalde tot 80 % CZV- verwijdering en 90 % SS- verwijdering bij een HRT van 3 uur. Het CEPS-UASB systeem zou in staat zijn het totale volume dat nodig is bij een klassieke UASB-reactor, te verminderen met een factor 0.4 om dezelfde efficiëntie te behouden.

De anaërobe co-digestie van het chemisch aangerijkt primair (CEP) slib tezamen met groenten-, fruit- en tuinafval (GFT) werd onderzocht in batchreactoren voor verschillende CEP-slib / GFT-afval verhoudingen (Hoofdstuk VII). De resultaten toonden dat de co-digestie van beide substraten compatibel is met elkaar. 50 tot 69 % VS destructie werd bekomen bij een belasting van 50 g TS l^{-1} reactor en een verblijftijd van 20 dagen. Verder maakte het CEP- slib de afbraak van GFT- afval mogelijk zonder een pH-correctie met $NaHCO_3$ te moeten uitvoeren. Dit was te wijten aan de hoge buffercapaciteit van het CEP- slib en de toename in alkaliniteit tijdens de werking van de reactoren door de omzetting van VFA in CH_4 en ammonificatie van de stikstof in het CEP- slib. Voor verdere toepassingen zal het gebruik van minimaal 25 % CEP- slib nodig zijn om een te hoge concentratie van zware metalen in het verteerde residu te vermijden.

Tot besluit wordt een geïntegreerd concept voor de anaërobe waterzuivering van huishoudelijk afvalwater voorgesteld, inclusief een vergisting van vast afval (Hoofdstuk VIII). De mogelijke voordelen van dit systeem zijn 1) terugwinnen van energie van organisch afval op een milieuvriendelijke manier, 2) verminderen van de negatieve effecten van SS in de UASB- reactor en 3) productie van een hoogkwalitatief effluent voor irrigatiedoeleinden. Deze voordelen dienen te worden bevestigd door het ontwerpen van een pilootsysteem.

REFERENCES

Agrawal L.K., Ohashi Y., Mochida E., Okui H., Ueki Y., Harada H. & Okashi, A. (1997) Treatment of raw sewage in a temperate climate using a UASB reactor and the hanging sponge cubes process. *Water Science and Technology* **36**, 433-440.

Ahel M., Giger W. & Koch M. (1994) Behavior of alkylphenol polyethoxylate surfactants in the aquatic environment-I. Occurrence and transformation in sewage treatment. *Water Research* **28**, 1131-1142.

Aiba S., Shoda M. & Nagatani M. (1968) Kinetics of product inhibition in alcohol fermentation. *Biotechnology and Bioengineering* **10**, 845-864.

Amann R., Lemmer H. & Wagner M. (1998) Monitoring the community structure of wastewater treatment plants: a comparison of old and new techniques. *FEMS Microbiology Ecology* **25**, 205-215.

APHA. (1992) *Standard methods for the examination of water and wastewater*, 18th ed. Amer. Public Health Assoc., Washington, D.C.

Arcand Y., Guiot S.R., Desrochers M. & Chavarie C. (1994) Impact of the reactor hydrodynamics and organic loading on the size and activity of anaerobic granules. *Chemical Engineering Journal* **56**, 23-35.

Atlas R.M. (1993) Handbook of microbiological media. Parks, L C (ed.). London-Tokyo: CRC press, 1079 p.

Atlas R.M., Horowitz A., Krichevsky M. & Bej A.K. (1991) Reponse of microbial populations to environmental disturbance. *FEMS Microbiology Ecology* **22**, 249-256.

Barbosa A. & Sant'Anna Jr.G.L. (1989) Treatment of raw domestic sewage in an UASB reactor. *Water Research* **23**, 1483-1490.

Berger M.R., Habs M., Jahn S.A.A. & Schmahl D. (1984) Toxicological assessment of seeds from *Moringa oleifera* and *Moringa stenopetala*, two highly efficient primary coagulants for domestic water treatment of tropical waters. *East African Medical Journal* **61**, 712-717.

Bitton G. (1999) Wastewater microbiology. Second Edition. Wiley-Liss, N.Y. 578 p.

Blumenkrantz N. & Asboe-Hansen G. (1973) New method for quantitative determination of uronic acids. *Analytical Biochemistry* **54**, 484-489.

Bogte J.J., Breure A.M., Van Andel J.G. & Lettinga G. (1993) Anaerobic treatment of domestic wastewater in small scale UASB reactors. *Water Science and Technology* **27** (9), 75-82.

Borda Biogas Manual. (1980) Biogas manual for the realization of biogas programmes, Bremen, FRG, 116 p.

Borzacconi L., López I. & Anido C. (1997) Hydrolysis constant and VFA Inhibition in acidogenic phase of MSW anaerobic dégradation. In *Proceedings of the 8th Internatinal Conference on Anaerobic Digestion*, document of the IAWQ, **1**, pp. 277-282, Sendai.

Boumansour B-E., El Ouarghi H., El Hamouri B. & Vasel J-L. (1998). Hydrodynamic and reaeration studies in a high rate algal pond. In *Proceedings of the 18th conference of the IAWQ*, Vancouver.

Brito A.G. & Melo L.F. (1997) A simplified analysis of reaction and mass transfer in UASB and EGSB reactors. *Environmental Technology* **18**, 35-44.

Britz T.J., Spangenberg G. & Venter C.A. (1994) Acidogenic microbial species diversity in anaerobic digesters treating different substrates. *Water Science and Technology* **30**, 55-61.

Brummeler E., Hulshoff Pol L.W., Dolfing J., Lettinga G. & Zehnder A.J.B. (1985) Methanogenesis in an upflow anaerobic sludge blanket reactor at pH 6 on an acetate-propionate mixture. *Applied and Environmental Microbiology* **49**, 1472-1477.

Buivid M.G., Wise D.L., Blanchet M.J., Remedios E.C., Jenkins B.M., Boydm W.F. & Pacey J.G. (1981) Fuel gas enhacement by controlled landfilling of municipal solid waste. *Resource Conservation* **6**, 3-20.

Burel C. & Trancart M.J.L. (1985) Mise au point d'un pilote de digestion anaérobie: conception et réalisation. *Tribune du Cebedeau* **494**, 27-43.

Castillo A., Cecchi F. & Mata-Alvarez J. (1997) A combined anaerobic-aerobic system to treat domestic sewage in coastal areas. *Water Science and Technology* **31**, 3057-3063.

Catunda P.F.C. & Van Haandel A.C. (1996) Improved applicability of waste stabilisation pond by pre-treatment in a UASB reactor. *Water Science and Technology* **33**, 147-156.

Champiat D. (1994) Application de la dégradation de la matière organique au traitement anaérobie des eaux résiduaires. In *Biologie des eaux*, Champiat D. & Larpent J.P. (eds.), pp. 159-181, Masson: Paris.

Chen X. (1996) USB septic tank combined with aerobic biofilter for domestic wastewater treatment. *M Sc thesis*, Ghent University.

Chernicharo C.A.L. & Borges J.M. (1997) Evaluation and start up of a full scale UASB reactor treating domestic sewage. Case study. In *Proceedings of the 8th International Conference on Anaerobic Digestion, May 25-29, 1997,* **2**, pp. 192-199. Sendai: document of the IAWQ, Pergamon press.

Chernicharo C.A.L. & Machado R.M.G. (1998) Feasibility of the UASB/AF system for domestic sewage treatment in developing countries. *Water Science and Technology* **38,** 325-332.

Chu C.W., Poon C.S. & Cheung R.Y.H. (1998) Characterization of raw sludge, chemically modified sludge and anaerobically digested sludge in Hong Kong. *Water Science and Technology* **38**(2), 25-32

Cisneros J.B. (1995) Wastewater reuse to increase soil productivity. *Water Science and Technology* **32**, 173-180.

Cisneros J.B. & Mejía C.A. (1997) Treatment of Mexico City wastewater for irrigation purposes. *Environmental Technology* **18**, 721-730.

Crowther R.F. & Harkness N. (1975) Anaerobic bacteria. In *Ecological Aspects of Used-water Treatment*, Curds C.R & Hawkes H.A. (eds.), pp. 65-91, Academic Press, London.

De Baere L. Rozzi A. & Verstraete W. (1984) Solubilisation of particulate organic matter as the rate-limiting step in anaerobic digestion loading. *Tribune du Cebedeau* **484** (37), 75-81.

De Baere L., Vaes H. & Verstraete W. (1982) Combined digestion of concentrated industrial wastes and municipal wastewater sludges. In *Proceedings of the International Symposium on Advances in Anaerobic Digestion*, Vinietgta G. (ed.), pp. 25-26, Mexico City.

De Baere L. & Verstraete W. (1984) Anaerobic fermentation of semi-solid and solid substrates. In *Anaerobic digestion and carbohydrate hydrolisis of waste*, Ferrero G.L., Ferranti M.P. & Naveau H. (eds.), pp. 195-208, Elsevier Applied Science Publishers, London.

De Baere L. & Verstraete W. (1982) Can the recent innovations in anaerobic digestion of wastewater be implemented in anaerobic sludge stabilization? In *Recycling International – Recovery of Energy and Material from Residues and Wastes*, Tomé-Kozmiensky K. J. (ed.), pp. 390-394, E. – Verlag Für Umwelttechniek, Freitag, Berlin.

De Beer D., O'Flaharty V., Thaveesri J., Lens P. & Verstraete W. (1996) Distribution of extracellular polysaccharides and flotation of anaerobic sludge. *Applied Microbiology and Biotechnology* **46**, 197-201.

De Man A.W.A., Rijs G.B.J., Lettinga G. & Van Starkenburg W. (1988a) Anaerobic treatment of sewage using a granular sludge bed UASB reactor. In *Proceedings of the 5th International Symposium on Anaerobic Digestion,* Tilche A. & Rozzi A. (eds.), pp.735-738. Bologna.

De Man A.W.A., Vander last A.R.M. & Lettinga G. (1988b) The use of EGSB and UASB anaerobic systems for low strength soluble and complex wastewaters at temperatures ranging from 8 to 30 °C. In *Proceedings of the 5th International Symposium on Anaerobic Digestion*, Hall E.R. & Hobson P.N. (eds.) pp. 197-209. Bologna.

De Sousa J.T. & Foresti E. (1996) Domestic sewage treatment in an upflow anaerobic sludge blanket – sequencing batch reactor system. *Water Science and Technology* **33**, 73-84.

De Velásquez M.T.O., Manero O.J., Cardoso J. & Martinez G. (1998) New polymeric coagulants tested in water and wastewater. *Environmental Technology* **19**, 323-330.

Dinopoulou G., Rudd T. & Lester J.N. (1988) Anaerobic acidogenesis of a complex wastewater : I. The influence of operational parameters on reactor performance. *Biotechnology and Bioengineering* **31**, 958-968.

Dixo N.G.H., Gambril M.P., Catunda P.F.C. & Van Haandel A.C. (1995) Removal of pathogenic organisms from the effluent of an upflow anaerobic digester using waste stabilization ponds. *Water Science and Technology* **31**, 275-284.

Doudina N.H., Panova E. & Petouhov M.P. (1991) Agro chemistry and use of fertilisers. Agropromizdat Edition, 400 p, In Russia.

Draaijer H., Maas J.A.W., Schaapman J.E. & Khan A. (1992) Performance of the 5 MLD UASB reactor for sewage treatment at Kampur, India. *Water Science and Technology* **25**(7), 123-133.

Dubourguier H.C., Buisson M.N., Tisser J.P., Prensier G. & Albagnac G. (1988) Structural characteristic and metabolic activities of granular methanogenic sludge on a mixed defined substrate. In *Granular anaerobic sludge : microbiology and technology,* Lettinga G.A., Zehnder A.J.B., Grotenhuis J.T.C., Hulshoff Pol L.W. (eds.) pp. 78. Wageningen : Pudoc, The Netherlands.

Edeline F. (1997a) Epuration biologique des eaux: Theorie et technologie des réacteurs, Lavoisier (Tec & Doc), 4ème edition, 303 p.

Edeline F. (1997b) Epuration physico-chimique des eaux: Theorie et technologie des réacteurs, Lavoisier (Tec & Doc), 4ème edition, 298 p.

El-Mamouni R., Leduc R. & Guiot S.R. (1998) Influence of synthetic and natural polymers on the anaerobic granulation process. *Water Science and Technology* **38,** 341-347.

El-Mamouni R., Leduc R. & Guiot S.R. (1997) Influence of the starting microbial nucleus type on the anaerobic granulation dynamics. *Applied Microbiology and Biotechnology* **47**, 189-194.

El-Mamouni R., Guiot S. R., Leduc R. and Costerton J. W. (1995) Charaterization of different microbial nuclei as potential precursors of anaerobic granulation. *Journal of Biotechnology* **39**, 239-249.

Elmitwalli T.A., Zandvoort M.H., Zeeman G., Bruning H. & Lettinga G. (1999) Low temperature treatment of domestic sewage in upflow anaerobic sludge blanket and anaerobic hybrid reactors. *Water Science and Technology* **39**, 177-186.

Elmitwalli T.A. (2000) Anaerobic treatment of domestic sewage at low temperature. *PhD thesis*. Wageningen University (Netherlands).

Eng S.C., Fernandes X.A. & Paskins A.R. (1986) Biochemical effects of administering shock loads of sucrose to a laboratory-scale anaerobic (UASB) effluent treatment plant. *Water Research* **20**, 789-794.

Espinosa A., Rosas L., Ilangovan K. & Noyala A. (1995) Effect of trace metals on the anaerobic degradation of volatile fatty acids in molasses stillage. *Water Science and Technology* **32**, 121-129.

Folkard G. & Sutherland J. (1994) *Moringa oleifera* a multipurpose tree. *Footsteps* **20**, 14 - 15.

Fresenius W., Quentin K.E. & Schneider W. (1988) Water analysis. A practical guide to physico-chemical, chemical and microbiological water examination and quality assurance. Springer-Verlag, Berlin, 804 p.

García E.P.A., Rodriguez R., Fernández N. & Fdz-Polanco F. (1998) Domestic sewage treatment with a two-stage anaerobic reactor. In *Preprints of the International Wimek Congress on Options for Closed Water Systems – Sustainable Water Management*, pp. 6-II, Wageningen University, The Netherlands.

Gassenschmidt U., Jany K.D., Tauscher B. & Niebergall H. (1995) Isolation and characterization of a flocculating protein from *Moringa oleifera* Lam. *Biochimica et Biophysica Acta* **1243**, 477-481.

Ghosh S. (1997) Anaerobic digestion for renewable energy and environmental restoration. In *Proceedings of the 8th International Conference on Anaerobic Digestion*, document of the IAWQ, **1**, pp. 9-16, Sendai.

Ghosh S., Henry M.P. & Klass D.L. (1980) Bioconversion of water hyacinth-coastal bermuda grass MSW-sludge blends to methane. *Biotechnology Bioengineering Symposium* **10**, 163-187.

Ghyoot W. & Verstraete W. (1997) Anaerobic digestion of primary sludge from chemical pre-precipitation. *Water Science and Technology* **36**(6-7), 357-365.

Gnanadipathy A. & Polprasert C. (1993) Treatment of domestic wastewater with UASB reactor. *Water Science and Technology* **27**, 195-203.

Gonçalves R.F., De Araújo V.L. & Chernicharo C.A.L. (1998) Association of UASB reactors and submerged aerated biofilters for domestic sewage treatment. *Water Science and Technology* **38**, 189-196.

Grabow W.O.K., Slabert J.L., Morgan W.S.G. & Jahn, S.A.A. (1985) Toxicity and mutagenicity evaluation of water coagulated with *Moringa oleifera* seeds preparations using fish, protozoan, bacterial, enzyme and ames salmonella assays. *Water SA* **11**, 9-14.

Grethlein H.E. (1978) Anaerobic digestion and membrane separation for the treatment of domestic waswater. *Journal Water Pollution Control Federation* **50**, 754-763.

Grin P.C., Roersma R.E. & Lettinga G. (1983) Anaerobic treatment of raw sewage at lower temperatures. In *Proceeding European Symposium on Anaerobic Wastewater Treatment (AWWT)*, Van Den Brink W.J. (ed.), pp. 335-347. Noordwijkerhout.

Grootaerd H., Liessens B. & Verstraete W. (1997) Effect of directly soluble and fibrous rapidly acidifying chemical oxygen demand and reactor liquid surface tension on granulation and sludge bed stability in upflow anaerobic sludge blanket reactors. *Applied Microbiology and Biotechnology* **48**, 304-310.

Gossett J.M., Mc Carty P.L., Wilson J.C. & Evans D.S. (1978) Anaerobic digestion of sludge from chemical treatment. *Journal Water Pollution Control Federation* **50**, 533-542.

Grusenmeyer S. & Pipyn. P (1987) Le traitement anaérobie en réacteur UASB: les résultats à la sucrerie d'Hollogne. *Tribune du Cebedeau* **528**(40), 19-26.

Gunerson C.G. & Stuckey D.C. (1986) Fundamentals of anaerobic digestion. In *Anaerobic Digestion: Principles and Practices for Biogas Systems*, World Bank Technical Report **49**, pp. 8-16.

Hamzawi N., Kennedy K.J. & Mc Lean D.D. (1998) Technical feasibility of anaerobic co-digestion of sewage sludge and municipal solid waste. *Environmental Technology* **19**, 993-1003.

Harada H., Uemura S. & Momonoi K. (1994) Interaction between sulphate-reducing bacteria and methane-producing bacteria in UASB reactors fed with low strength wastes containing differents levels of sulfate. *Water Research* **28**, 355-367.

Harleman D., Morrisey S. & Murcott S. (1991) The case for using chemically enhanced primary treatment in a new cleanup for Boston Harbor. Journal of the Boston Society, April, 69-84.

Henze M., Harremoës P., La Cour Jansen J. & Arvin E. (2000) Wastewater treatment. Biological and chemical processes. Springer-Verlag, Germany, 385 p.

Hoban D.J. & Van der Berg L. (1979) Effect of iron conversion of acetic acid to methane during methanogenic fermentations. *Journal of Applied Bacteriology* **47**, 153-159.

Howgrave-Graham A.R., Isherwood H.A. & Wallis F.M. (1994) Evaluation of two upflow anaerobic digesters purifying industrial wastewaters high in organic matter. *Water Science and Technology* **29**, 225-229.

Hulshoff Pol L.W., Heijnekamp K., Lettinga G. (1988) The selection pressure as a driving force behind the granulation of sludge. In *Granular anaerobic sludge : microbiology and technology,* Lettinga G.A., Zehnder A.J.B., Grotenhuis J.T.C., Hulshoff Pol L.W. (eds.), pp. 153. Wageningen : Pudoc, The Netherlands.

Hulshoff Pol L.W., Euler H., Eitner A. & Grohganz D. (1997) GTZ sectorial project promotion of anaerobic technology for the treatment of municipal and industrial sewage and wastes. In *Proceedings of the 8th international Conference on Anaerobic Digestion, May 25-29, 1997,* **2**, pp. 285-292. Sendai: document of the IAWQ, Pergamon press.

Hulshoff Pol L.W., Heijnekamp K. & Lettinga G. (1988) The selection pressure as a driving force behind the granulation of sludge. In *Granular anaerobic sludge : microbiology and technology*, Lettinga G., Zehnder A.J.B., Grotenhuis J.T.C. & Hulshoff Pol L.W. (eds.), pp. 153, Pudoc, Wageningen, The Netherlands.

Hwu C-S. (1997) Enhancing anaerobic treatment of wastewaters containing oleic acid. *PhD thesis*. Wageningen University (The Netherlands).

Imai T., Ukita M., Lui J., Sekine M., Nakanishi H. & Fukagawa M. (1997) Advanced startup of UASB reactors by adding of water absorbing polymer. *Water Science and Technology* **36**, 399-406.

Jenkins D., Richard M.G. & Daigger G.T. (1993) Manual on the causes and control of activated sludge bulking and foaming. London-Tokyo: Lewis Publishers. 193 p.

Kalker T.J.J., Maas J.A.W. & Zwaag R.R. (1999) Transfer and acceptance of UASB technology for domestic wastewater : two case studies. *Water Science and Technology* **39**, 219-226.

Kalogo Y. (1995) Mesure de l'influence de paramètres physico-chimique sur la coagulation d'une eau brute de turbidité moyenne 200 NTU. Maîtrise de 3ème Cycle en Sciences de l'Environnement, Fondation Universitaire Luxembourgeoise, Arlon, Belgique.

Kalogo Y. & Verstraete W. (1999) Development of anaerobic sludge bed (ASB) reactor technologies for domestic wastewater treatment - motives and perspectives. *World Journal of Microbiology and Biotechnology* **15**, 523-534.

Karlson I. (1985) Chemical phosphorus removal in combinaison with biological treatment. Hahn H.H. & Klute R. (eds.), pp. 261-271, Lisbon.

Kato T.M., Field J.A., Versteeg P. & Lettinga G. (1994) Feasibility of expanded granular sludge bed reactors for the anaerobic treatment of low strength soluble wastewaters. *Biotechnology and Bioengineering* **44**, 469-479.

Kato T.M., Field J.A. & Lettinga G. (1997) The anaerobic treatment of low strength wastewaters in UASB and EGSB reactors. *Water Science and Technology* **36**, 375-382.

Kerharo J. (1974) *Moringa oleifera*. In *la pharmacopée sénégalaise traditionnelle- plantes médicinales et toxiques*, Vigot-frères (eds.), pp. 598-602, Paris.

Kersters I., Van Vooren L., Huys G., Janssen P., Kersters K. & Verstraete W. (1995) Influence of temperature and process technology on the occurrence of *Aeromonas* species and hygienic indicator organisms in drinking water production plants. *Microbial Ecology* **30**, 203-218.

Kong Z., Liessens B. & Verstraete W. (1995) Effects of RACOD and reactor liquid surface tension on granulation and sludge bed stability in UASB reactors. In *Proceedings of the 9th Forum for Applied Biotechnology.* Ghent University. **60/4b**. pp. 2187-2194.

Kooymans J.L. & Van Velsen A.F.M. (1986) Application of the UASB process for treatment of domestic sewage under sub-tropical conditions, the cali case. In *Proceedings Anaerobic Treatment a Grow up Technology (AQUATECH 86), September 15-19, 1986*, s-Gravelandseweg 284-296 (ed.), 3125 BK Schiedam The Netherlands. pp. 423-436.

Lettinga G. (1995) Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek* **67**, 3-28.

Lettinga G. (1996) Sustainable integrated biological wastewater treatment. *Water Science and Technology* **33**, 85-98.

Lettinga G., De Man A., Vanderlast A.R.M., Wiegant W., Van Knippenber K., Frijns J. & Van Buuren J.C.L. (1993) Anaerobic treatment of domestic sewage and wastewater. *Water Science and Technology* **27**(9), 67-73.

Lettinga G. & Hulshoff Pol L.W. (1991) UASB-process design for various types of wastewater. *Water Science and Technology* **24**, 87-107.

Lettinga G., Roersma R., Grin P., De Zeeuw W., Hulshof Pol L.W., Van Velsen L., Hobman S. & Zeeeman G. (1981) Anaerobic treatment of sewage and low strength wastewaters. In *Proceedings of the 2nd International Symposium on Anaerobic Digestion*, eds Hughes D.E., Stafford D.A., Wheatley B.I., Baader W., Lettinga G., Nyns E.J., Verstrate W. & Wentworth R.L. (eds.), pp. 271-291, Travemünde, Elsevier Biomedical Press: Amsterdam.

Lettinga G., Roersma R. & Grin P. (1983) Anaerobic treatment of raw domestic sewage at ambient temperature using a granular bed UASB reactor. *Biotechnology and Bioengineering* **25**, 1701-1723.

Lettinga G., Van Velsen A.F.M., Hobma S.W., De Zeeuw W. & Klapwijk A. (1980) Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering* **22**, 699-734.

Levenspiel O. (1972) Chemical reaction engineering. 2nd Edition. John Wiley & Sons, New York.

Levine A.D., Tchobanoglous G. & Asano T. (1985) Characterization of the size distribution of contaminants in wastewater: Treatment and reuse implications. *Journal Water Pollution Control Federation* **57**(7), 805-816.

Mac Leod F.A., Guiot S.R., Costerton W.J. (1990) Layered structure of bacterial aggregates produced in an uplow anaerobic sludge bed and filter reactor. *Applied and Environmental Microbiology* **56**, 1598-1607.

Mah A.R. (1983) Interactions of methanogens and non-methanogens in microbial ecosystems. In *Proceedings of the 3rd International Symposium on Anaerobic Digestion*, Boston, Printed by Evans and Faulkner Incorporated Watertown, Massachussetts, 13-22.

Maqueda C., Pérez-Rodríguez J.L, Lebrato J. (1998) An evaluation of clay minerals as support materials in anaerobic digesters. *Environmental Technology* **19**, 811-819.

Mathisen B. (1997) Anaerobic digestion as an integrated treatment for source separated waste. In *Proceedings of the 5th FAO/SREN-WORKSHOP on Anaerobic Conversions for Environmental Protection, Sanitation and Re-use of Residues*, Verstraete W. (ed.), pp. 257-261, Ghent University.

Mergaert K., Vanderhaegen B. & Verstraete W. (1992) A review. Application and trends of anaerobic pre-treatment of municipal wastewater. *Water Research* **26**, 1025-1033.

Monroy O., Famá G., Meraz M., Montoya L. & Macarie H. (2000) Anaerobic digestion for wastewater treatment in Mexico. State of the technology. *Water Research* **34**, 1803-1816.

Monroy O., Noyola A., Ramirez F. & Guiot J.P. (1988) Anaerobic digestion of water hyacinth as a highly efficient treatment process for developing countries. In *Proceedings of the 5th International Conference on Anaerobic Digestion*, Hall E.R. & Hobson P.N. (eds.), pp. 347-351. Bologna.

Mossakowska A., Hellström B.G. & Hultman B. (1998) Strategies for sludge handling in the Stockhlom region. *Water Science and Technology* **38**(2), 111-118.

Muyibi S.A, Evison L.M. (1995) *Moringa oleifera* seeds for softening hardwater. *Water Research* **29**, 1099-1105.

N'Dabigengesere A. & Narasiah S.K. (1998a) Quality of the water treated by coagulation using *Moringa oleifera* seeds. *Water Research* **32**, 703-710.

N'Dabigengesere A. & Narasiah S.K. (1998b) Use of *Moringa oleifera* seeds as a primary coagulant in wastewater treatment. *Environmental Technology* **19**, 789-800.

N'Dabigengesere A. & Narasiah S.K. (1996) Influence of operating parameters on turbidity removal by coagulation with *Moringa oleifera* seeds. *Environmental Technology* **17**, 1103-1112.

N'Dabigengesere A., Narasiah S.K. & Talbot B.G. (1995) Active agents and mechanism of coagulation of turbid waters using *Moringa oleifera*. *Water Research* **29**, 703-710.

Noike T., Endo G., Chang J.E., Yaguchi J.I. & Matsumoto J.I. (1985) Characteristics of carbohydrate degradation and the rate-limiting step in anaerobic digestion. *Biotechnology and Bioengineering* **27**, 1482-1489.

Nordberg A. & Edström M. (1997) Co-digestion of ley crop silage, source-sorted municipal solid waste and municipal sewage sludge. In *Proceedings of the 5th FAO/SREN-WORKSHOP on Anaerobic Conversions for Environmental Protection, Sanitation and Re-use of Residues*, Verstraete W. (ed.), pp. 162-171, March 1997, Ghent University.

Noyola A. & Morena G. (1994) Granule production from raw activated sludge. *Water Science and Technology* **30**(12), 339-346.

O'Flaherty V., Lens P.N.L., de Beer D. & Colleran E. (1997) Effect of feed composition and upflow velocity on aggregate characteristics in anaerobic upflow reactors. *Applied Microbiology and Biotechnology* **47**, 102-107.

Okuda T., Baes A.U., Nishijima W. & Okada M. (1999) Improvement of extraction method of coagulation active components from *Moringa oleifera* seed. *Water Research* **33**, 3373-3378.

Oleszkiewicz J.A. & Poggi M.V. (1997) High-solids anaerobic digestion of mixed municipal and industrial waste. *Journal of Environmental Engineering* **23**(11), 1087-1092.

Olsen A. (1987) Low technology water purification by bentonite clay and *Moringa oleifera* seeds floculation as performed in Sudanese villages : effect on *Schistosoma mansoni cercariae*. *Water Research* **21**, 517-522.

Ooghe W. (1983) On the influence of hydrogen peroxide on the amino acid composition of foodstuffs. In *Proceedings of the European Conference on Food Chemistry*, Federation European of Chemistry Societies, WPFC (ed.), pp. 315-321, Rome.

Pette K.C. & Versprille A.I. (1981) Application of the UASB – concept for wastewater treatment. In *Proceedings of the 2nd International Symposium on Anaerobic Digestion*, Hughes D.E., Stafford D.A., Wheatley B.I., Baader W., Lettinga G., Nyns E.J., Verstraete W. & Wentworth R.L. (eds.), pp. 121-133, Travemünde, Elsevier Biomedical Press: Amsterdam.

Pielou E.C. (1966) The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology* **13**, 131-144.

Pipyn P., Derycke D. & Defour D. (1994) Sludge production and treatment as important factors in the cost/benefit calculation in the choice of the most appropriate wastewater treatment system. In *Proceedings of the 8th Forum for Applied Biotechnology*. Ghent University. **59/4a**. pp. 1951-1958.

Pollard S.J.T., Thompson F.E. & Mc Connachie G.L. (1995) Microporius carbons from *Moringa oleifera* husk for water purification in less developed countries. *Water Research* **29**, 337-347.

Rebac S. (1998) Psychrophilic anaerobic treatment of low strength soluble wastewaters. *PhD thesis*. Wageningen University (Netherlands).

Rozzi A. & Verstraete W. (1981) Calculation of active biomass and sludge production vs waste composition in anaerobic contact processes. *Tribune du Cebedeau*, **455**(34), 421-427.

Ross W.R., Novella P.H., Pitt A.J., Lund P., Thomson B.A., King P.B. & Fawcett K.S. (1992) Anaerobic digestion of wastewater sludge: operation guide, Water Research Commission, Pretoria, South Africa. 182 p.

Sayed S.K.I.A. & Fergala M.A.A. (1995) Two stage UASB concept for treatment of domestic sewage including sludge stabilization process. *Water Science and Technology* **32**, 55-63.

Schellinkhout A. (1993) UASB technology for sewage treatment : Experience with a full-scale plant and its applicability in Egypt. *Water Science and Technology* **27**, 173-180.

Schellinkhout A. & Collazos C.J. (1992) Full-scale application of the UASB technology for sewage treatment. *Water Science and Technology* **25**, 159-166.

Schellinkhout A., Jakm F.F.G. M. & Forero G.E. (1988) Sewage treatment : the anaerobic way is advancing in Colombia. In *Proceedings of the 5th International Symposium on Anaerobic Digestion*, Hall, E.R. & Hobson, P.N. (eds.), pp. 767-770. Bologna.

Schellinkhout A., Lettinga G., Van Velsen L., Kooijmans J.L. & Rodriguez G. (1985) The application of the UASB reactor for the direct treatment of domestic wastewater under tropical condition. In *Proceedings of the Seminar/Workshop on Anaerobic treatment of sewage*, Switzenbaum (ed.), pp. 259-276, Boston, Massachussets.

Schellinkhout A. & Osorio E. (1994) Long term experience with the UASB technology for sewage treatment on large scale. In *Proceedings of the International Symposium on Anaerobic Digestion*, Printed by: RSA Litho(Pty)Ltd, pp. 251-252, Cape Town.

Schmidt J.E. & Ahring B.K. (1994) Extracellular polymers in granular sludge from different upflow anaerobic sludge blanket (UASB) reactors. *Applied Microbiology and Biotechnology* **42**, 457-462.

Schmidt J.E. & Ahring B.K. (1996) Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors. *Biotechnology and Bioengineering* **49**, 229-246

Schultz C.R. & Okun D. (1984) Surface water treatment for communities in developing countries. Wiley, New York.

Seghezzo L., Zeeman G., Van Lier J., Hamelers H.V.M. & Lettinga G. (1998) A review. The anaerobic treatment of sewage in UASB and EGSB reactors. *Bioresource Technology* **65**, 175-190.

Shao Y.J., Lui A., Jenkis D., Wada F. & Cross J. (1993) Advanced primary treatment: an alternative to biological secondary treatment. The city of Los Angeles (CA) Hyperion treatment plant experience. In *Proceedings of the 66th Annual Conference and Exposition*, pp. 181-193, Anahein, California.

Shelton D.R. & Tiedje J.M. (1984) General method for determining anaerobic biodegradation potential. *Applied and Environmental Microbiology* **47**, 850-857.

Shereif M.M., El-S Easa M., El-Samra M.I. & Nancy K.H. (1995) A demonstration of wastewater treatment for reuse applications in fish production and irrigation in Suez, Egypt. *Water Science and Technology* **32**, 137-144.

Souza M.E., Fuzaro G. & Polegato A.R. (1992) Thermophilic anaerobic digestion of vinasse in pilot plant UASB reactor. *Water Science and Technology* **25**(7), 213-222.

St Joly C. (1992) Three years of performances control and process monitoring in an industrial plant for municipal solid waste treatment by anaerobic digestion. In *Proceedings of the International Symposium on Anaerobic Digestion*, Cecchi F., Mata-Alvarez J., & Pohland F.G (eds.), pp. 546-550, IAWPRC, ISWA, Università Venezia, Universitat de Barcelona (Sponsors), Venice.

Szikrist G., Frostell B., Norrma J. & Bergström R. (1988) Pilot-scale anaerobic digestion of municipal solid waste after a novel pre-treatment. In *Proceedings of the International Symposium on Anaerobic Digestion*, Tilche A. & Rozzi A. (eds.), pp. 375-382, Printed by Monduzzi editore, Bologna.

Takaski A. (1998) Wastewater reclamation and reuse. Takaski editor, **10**, 1528 p.

Tare V., Mansoor A.M. & Jawed M. (1997) Biomethanation in domestic and industrial waste treatment- an Indian scenario. In *Proceedings of the 8th international Conference on Anaerobic Digestion, May 25-29, 1997,* **2**, pp. 255-262. Sendai: document of the IAWQ, Pergamon Press.

Tchobanogous G. & Burton F.L. (1991) Wastewater engineering. Treatment disposal reuse. *McGraw-Hill International Editions*, Third edition, NY, 1334 p.

Tchobanoglous G., Thiesen H. & Vigil S. (1993) Integrated solid waste management-engineering principles and management issues. *Mc Graw-Hill, inc*, 978 p.

Ten Brummeler E., Koster I.W. & Zeevalkink J.A. (1988) Dry digestion of the organic fraction of municipal soild waste in a batch process. In *Proceedings of the 5Th International Symposium on Anaerobic Digestion*, Bologna, Hall E.R & Hobson P.N. (eds.), pp. 335-344, Pergamon Press.

Thaveesri J. (1995) Granulation and stability in upflow anaerobic sludge bed reactors in relation to substrates and liquid surface tension. *PhD thesis*. Ghent University.

Thaveesri J., Daffonchio D., Liessens B., Vandermeren P. & Verstraete W. (1995a) Granulation and sludge bed stability in upflow anaerobic sludge bed reactors in relation to surface thermodynamics. *Applied and Environmental Microbiology* **61**, 3681-3686.

Thaveesri J., Liessens B. & Verstraete W. (1995b) Granular sludge growth under different reactor liquid surface tensions in lab-scale upflow anaerobic sludge blanket reactors treating wastewater from sugar beet processing. *Applied Microbiology and Biotechnology* **43**, 1122-1127.

Trousset P. & Morin J.F. (1994) Mathématiques pour les sciences de la vie – probabilité et statistiques. Ediscience International, Paris, 345 p.

Valcke D. & Verstraete W. (1983) A practical method to estimate the acetoclastic methanogenic biomass in anaerobic sludges. *Journal Water Pollution Control Federation* **55**, 1191-1195.

Vanderhaegen B., Ysebaert E., Pavere K., Van Wambeke M., Peeters T., Panic V., Vandenlangenbergh V. & Verstraete W. (1992) Acidogenesis in relation to in-reactor granule yield. *Water Science and Technology* **25**, 21-30.

Vander Last A.R.M. & Lettinga G. (1992) Anaerobic treatment of domestic sewage under moderate climatic (Dutch) conditions using upflow reactors at increased superficial velocities. *Water Science and Technology* **25**, 167-178.

Van Haandel A. (1994) Influence of the digested COD concentration on the alkalinity requirement in anaerobic digesters. *Water Science and Technology* **30**(8), 23-34.

Van Haandel A.C. & Catunda P.F.C. (1997) Application and perspectives of the anaerobic wastewater treatment in Latin America. In *Proceedings of the 5th FAO/SREN Workshop on Anaerobic Conversions for Environmental Protection, Sanitation and Re-use of Residues*, Verstraete W. (ed.), pp. 1-26, March 1997, Ghent University, Belgium.

Van Haandel A.C., Catunda P.F.C. & Lettinga G. (1996) The UASB reactor for industrial and domestic wastewater treatment. In *Environmental Waste Management: A European perspective*. Horan N. (ed.), pp. 2-29, John Wiley & Sons Ltd, London.

Van Haandel A.C. & Lettinga G. (1994) Anaerobic Sewage Treatment. A Practical Guide for Regions with a Hot Climate. John Wiley & Sons, New York, 212 p.

Van Der Steen P., Brenner A., Van Buuren J. & Oron G. (1999) Post-treatment of UASB reactor effluent in an integrated duckweed and stabilization pond system. *Water Research* **33**, 615-620.

Verstraete W., De Baere L. & Rozzi A. (1981) Phase separation in anaerobic digestion: motives and methods. *Tribune du Cebedeau* **453-454**, 367-375.

Verstraete W. & Vandevivere P. (1999) A review. Broader and newer applications of anaerobic digestion. *Critical Reviews in Environmental Science and Technology* **28**, 151-173.

Vieira S.M.M. & García Jr.D. (1992) Sewage treatment by UASB-reactor. Operation results and recommendations for design and utilization. *Water Science and Technology* **25**, 143-157.

Vieira S.M.M. (1988) Anaerobic treatment of domestic sewage in Brazil. Research results and full-scale experience. In *Proceedings of the 5th International Conference on Anaerobic Digestion*, Hall E.R. & Hobson P.N. (eds.), pp. 185-196, Bologna, Pergamon Press: London.

Vieira S.M.M., Carvalho J.L., Barijan F.P.O. & Rech C.M. (1994) Application of the UASB technology for sewage treatment in small community at Sumare, São Paulo State. *Water Science and Technology* **30**, 203-210.

Vieira S.M.M. & Souza M.E. (1986) Development of technology for the use of the UASB reactor in domestic sewage treatment. *Water Science and Technology* **18**, 109-121.

Visser A., Gao Y. & Lettinga G. (1992) Anaerobic treatment of synthetic sulfate-containing wastewater under thermophilic conditions. *Water Science and Technology* **25**(7), 193-202.

Wang K. (1994) Integrated Anaerobic and Aerobic Treatment of Sewage. *PhD thesis*. Wageningen University (The Netherlands).

Wang K., Vander Last A.R.M. & Lettinga G. (1997) The hydrolysis upflow sludge bed (HUSB) and the expanded granular sludge blanket (EGSB) reactors process for sewage treatment. In *Proceedings of the 8th International Conference on Anaerobic Digestion, May 25-29, 1997*, **3**, pp. 301-304. Sendai: document of the IAWQ, Pergamon Press.

Waweru M. (1997) Integrated treatment of mixed domestic solid waste. *Master Thesis*, Ghent University, 53 p.

Weiland P. (1997) Co-digestion of agricutural, industrial and municipal waste. In *Proceedings of the 5th FAO/SREN-WORKSHOP on Anaerobic Conversions for Environmental Protection, Sanitation and Re-use of Residues*, Verstraete W. (ed.), pp. 42-52, March 1997, University of Ghent, Belgium.

Wiegant W.M. & De Man A.W.A. (1986) Granulation of biomass in thermophilic upflow anaerobic sludge blanket reactors treating acidified wastewaters. *Biotechnology and Bioengineering* **28**, 718-727.

Widdel F. (1988) Microbiology and ecology of sulfate and sulfur-reducing bacteria. In *Biology of Anaerobic Microorganisms*, Zehnder, A.J.B. (eds.), pp. 469-586. *NY: Wiley*.

Wiegel J. (1990) Temparature spans for growth: hypothesis and discussion. *FEMS Microbiology Review* **75,** 155-170.

Wirtz R.A. & Dague R.R. (1996) Enhancement of granulation and start up in the anaerobic sequencing batch reactor. *Water Environment Research* **85,** 883-892.

World Health Organization. (1989) Health guidelines for the use of wastewater in agriculture and aquaculture. Report of a WHO Scientific Group, Geneva, Switzerland.

Wu W.M., Jain M.K., Conway de Macario E., Thiele J.H. & Zeikus J.G. (1992) Microbial composition and characterization of prevalent methanogens and acetogens isolated from syntrophic methanogenic granules. *Applied Microbiology and Biotechnology* **38**, 282-292.

Wu W-M., Jain M.K. & Zeikus G. (1996) Formation of fatty acid-degrading anaerobic granules by defined species. *Applied and Environmental Microbiology* **62**, 2037-2044.

Wu W.M., Thiele J.H., Jain M.K., Pankratz H.S., Hickey R.F. & Zeikus J.G. (1993) Comparison of rod-versus filament-type methanogenic granules: microbial population and reactor performance. *Applied Microbiology Biotechnology* **39**, 795-803.

Yoda M., Kitagawa M. & Miyaji Y. (1987) Long term competition between sulphate reducing bacteria and methane producing bacteria in anaerobic biofilm. *Water Research* **21**, 1547-1556.

Zeeman G. & Lettinga G. (1999) The role of anaerobic digestion of domestic sewage in closing the water and nutrient cycle at community level. *Water Science and Technology* **39**, 187-194.

Zeikus J.G. (1979) Microbial populations in digesters. In *Proceedings of the First International Symposium on Anaerobic Digestion*, Stafford D.A., Wheatley B.I. & Hughes D.E. (eds.), pp. 61-89, Applied Science Publishers Ltd: London.

Zellner G., Gereke M., Conway de Macario E. & Diekman H. (1991) Population dynamics of biofilm development during start-up of a butyrate-degrading fluidized bed reactor. *Applied Microbiology and Biotechnology* **36**, 404-409.

CURRICULUM VITAE

Youssouf Kalogo was born in 1969-03-18 in Bouaké (Republic of Côte d'Ivoire). He originates from Koro, Department of Touba (North-West of Côte d'Ivoire). He is currently engaged with Miss Bara Djata Coulibaly.

ADDRESS

21 BP 106 Abidjan 21 Côte d'Ivoire
E-mail: Youskalo@hotmail.com

EDUCATION

1997-2000	University of Ghent **Ph.D. student in Environmental Technology**
1997-1999	Faculté des Sciences Agronomiques de Gembloux **Sanitary Engineer**, Great distinction
1995-1996	Fondation Universitaire Luxembourgeoise **D.E.A in Environmental Science**, Distinction
1994-1995	Fondation Universitaire Luxembourgeoise **Master in Environmental Science**, Distinction
1990-1994	University of Côte d'Ivoire (Abidjan-Cocody) **Master in Physics and Chemistry**
1983-1989	Secondary School (CEG Koko-Bouaké / Lycée Sakassou) **Baccalaureate** (Mathematics and Physics)

INTERNATIONAL PUBLICATIONS

1. **Kalogo Y. & Verstraete W.** (1999) Development of anaerobic sludge bed (ASB) reactors technologies for domestic wastewater treatment: motives and perspectives. *World Journal of Microbiology and Biotechnology* **15**, 523-534.
2. **Kalogo Y. & Verstraete W.** (2000) Technical feasibility of the treatment of domestic wastewater by a CEPS-UASB system. *Environmental Technology* **21**, 55-65.
3. **Hammes F., Kalogo Y. & Verstraete W.** (2000) Anaerobic digestion technologies for closing the domestic water, carbon and nutrient cycles. *Water Science and Technology* **41**(3), 203-211.
4. **Kalogo Y., Rosillon F., Hammes F. & Verstraete W.** (2000) Effect of a water extract of *Moringa oleifera* seeds on the hydrolytic microbial species diversity of a UASB reactor treating domestic wastewater. *Letters in Applied Microbiology* **31**, 259-264.
5. **Kalogo Y., Mbouche J-H. & Verstraete W.** (2000) Physical and Biological performance of a self-inoculated UASB reactor treating raw domestic sewage. *Journal of Environmental Engineering* (in press).
6. **Kalogo Y., Séka M.A. & Verstraete W.** (2000) Enhancing the start-up of a UASB reactor treating domestic wastewater by adding a water extract of *Moringa oleifera* seeds. *Applied Microbiology and Biotechnology* (in press).
7. **Kalogo Y. & Verstraete W.** (2000). La digestion anaérobie des déchets solides. In *La Gestion de l'Environment dans les Industries Agro-alimentaires*, Chapitre VIIIb, Moletta R. (ed.), Tec & Doc, Lavoisier, Collection Sciences & Techniques Agro-alimentaires. (In press).
8. **Kalogo Y. & Verstraete W.** (2001) Potentials of anaerobic treatment of domestic sewage under temperate climate conditions. In Decentralised Sanitation and Reuse, Chapter 10, Lens P. & Lettinga G. (eds.), IWA publisher. (In press).

9. **Kalogo Y., Biey M.E. & Verstraete W.** Anaerobic co-digestion of VFG-waste and CEP-sludge without addition of external alkalinity. Submitted to *Journal of chemical Technology and Biotechnology*.
10. **Kalogo Y. & Verstraete W.** Granulation in upflow anaerobic reactor treating domestic sewage - effect of reactor hydrodynamics and feed composition. Submitted to *Water Research*.

PROFESSIONAL RECORD

Research and teaching assistant at the Faculté des Sciences Agronomiques de Gembloux since October 2000: Program of Sanitary Engineering.

CONTRIBUTION TO CONGRESSES

1. Second International Symposium on Anaerobic Digestion of Solid Waste, June 15-17, 1999, Barcelona, Spain, Paper presentation.
2. Thirteenth Forum for Applied Biotechnology (FAB), September 22nd and 23rd, 1999, Gent, Belgium, Poster presentation.
3. International Conference on "Anaerobic Digestion: Opportunities and Solutions for renewable energy and nutrient recycle in the farming and food-processing sectors" November 4th and 5th, 1999, Galway, Ireland, Poster presentation.